



# FEDERATION PROCEEDINGS

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## THE AMERICAN PHYSIOLOGICAL SOCIETY, INC

FIFTY SEVENTH ANNUAL MEETING

Atlantic City, New Jersey, March 15, 16, 17, 18, 19, 1948

(For possible corrections in any of the following abstracts see the next issue)

**Cold tolerance and old immersion in infant rats** E F ADOLPH *Dept of Physiology, The University of Rochester, Rochester, N Y* Rats aged 0 to 17 days recovered after refrigeration, either in oxygen (Fairfield, this issue) or in air, with body temperatures from 3° to 10°C. Such temperatures were tolerated for 2.5 hours, but not for 3 hours. Yet the rats did not tolerate temperatures below 15° when immersed in water to the shoulders. Evidently body temperature was not the only factor in survival of infants. Cold exposure, continued in an atmosphere of nitrogen after the breathing had ceased from cold, allowed no recovery. Surrounded by a rubber jacket held away from the trunk, the infants survived colonic temperatures of 6° while environed by water of 5°. Immersion up to the hips gave similar diminutions of colonic and mouth temperatures, with survival. Such cooling could be followed by immersion to the shoulders for one hour once breathing had ceased, with survival. But gradual cooling of water to 8° while chests were immersed was lethal. Evidently the damage was imposed during the cooling period. Upon rewarming, whether slowly in air or rapidly in water of 35°, the heart regularly resumed beating. Whenever reflexes and breathing also were regained, survival was permanent. Part of the lethal damage may be due to hydrostatic pressure about the chest, and part to local anoxia. Survival of infant rats below 15° depends only in part on their well-known tolerance of anoxia, whereas in hibernants cardiorespiratory functions persist at all temperatures compatible with survival.

**Lethality of cold immersion in rats** E F ADOLPH *Dept of Physiology, The University of Rochester, Rochester, N Y* Does failure to survive exposure to cold depend predominantly upon the low temperature to which certain tissues are exposed? Can that temperature be modified? Albino rats were cooled in various ways while their colonic (and sometimes esophageal) temperatures were being measured by thermocouples.

Immersion to the shoulders in water of 14.8°C for 2 hours was lethal for half of individuals, and none survived in 14.0°. Colonic temperatures at 2 hours were within one Centigrade degree of the water temperature. Reflexes and breathing gradually disappeared, if any remained the rats recovered when placed into air of 25°. Especially when cooling was rapid, reflex and cardiac activ-

ities disappeared some time after lethal temperatures were passed, since, in cold, functional breakdowns are delayed. Slow cooling of rats covered by rubber jackets and surrounded by water for 5 hours gave the same lethal temperatures as above. When immersed, uncovered, up to the hips and abdomen, the rats cooled equally slowly, and survived lower colonic temperatures (12° to 14° after 5 hours), but esophageal temperatures were one to three degrees higher. Many individuals died after rewarming with delays of hours or a day, thus they survived only temporarily when colonic temperatures went below 14°C. Median lethal temperatures were not modified by sex, age above 30 days, administrations of glucose or of cortin, deprivations of food or of water, nor repetition of the brief cold exposure. No way is known of securing continued survival of unanesthetized mature rats, after the chest and head have been cooled below 14°.

**The reaction caused by the intravenous injection of salmine sulfate in dogs** J GARROTT ALLEN and WILLADENE EGNER (by invitation) *From the Department of Surgery of the University of Chicago* Salmine sulfate may be used to control hemorrhage from overdosage of heparin because it forms an inactive salt with heparin. This substance has been said to be toxic, when given intravenously, producing an anaphylactoid reaction. Since this substance agglutinates the red blood cells in certain mammalian species and causes fibrinogen precipitation, the anaphylactoid reaction has been ascribed to the "plugging" of the peripheral vascular bed. Experiments on dogs are presented which show that animals given 5 to 15 mg of salmine sulfate per kg of body weight, intravenously, caused an anaphylactoid reaction. Dyspnea and a sharp fall in the arterial blood pressure occurred within two minutes after the injection of salmine. Dyspnea persisted only two or three minutes, but the blood pressure recovered slowly over a period of 30 to 40 minutes. A second injection of the same quantity of salmine sulfate made an hour after the first injection was not followed by any appreciable fall of the arterial blood pressure and dyspnea did not occur. When the animals were given the same amount of salmine in the same manner again one week later, the phenomenon of tachyphylaxis was again observed. Coagulation studies, red cell counts, hematocrit readings, and platelet counts made, showed no significant change after the injections. Leukopenia did occur but the white blood

count returned to normal, as the blood pressure was restored to normal. Following the second injection, when the animal showed no obvious systemic reaction, leukopenia did not occur. At the dosage used, no change in the fibrinogen content was noted after either injection of salmine. Five to ten times the amount employed here was necessary to produce fibrinogen precipitation.

**A method for estimating traces of T-1824 by combination with cellophane.** THOMAS H. ALLIN and PETER D. ORAHOVATS (by invitation) *Department of Physiology, College of Physicians and Surgeons, Columbia University*. Traces of the blue dye, T-1824, can be estimated quantitatively in the form of its combination with Cellophane. Ninety-eight per cent of the dye present in a 25 ml. solution (0.48% dye diluted 1:10,000 with 0.9% NaCl) is bound on 6.5 mg. of Cellophane foil immersed in the solution for 24 hours at 65°C. The optical density of the dye on the strip is 0.372 at 635  $\mu$ . When the concentration is doubled the Cellophane optical density increases 2.08 times. The product of the weight of the Cellophane and the optical density is directly related to the concentration of dye over the dilution range of 0 to 5 parts in 10,000. The homogeneity of staining and the spectral properties of the dye-Cellophane have been studied as a function of time, volume of solution, temperature, pH, concentrations of NaCl and Aerosol OT. This method appears to be selective, reasonably accurate and can be used in urine or blood serum.

T-1824 appears in dog urine within 30 minutes after intravenous injection of the amounts commonly used for blood volume determinations. During the ensuing 24 hours 2 parts in one thousand of the injected dye are excreted by the kidney. This value decreases to 3 parts in ten thousand on the second day.

Five drops of dye-tinged dog blood yield Cellophane values which agree well with those obtained by direct spectrophotometric analysis of the serum.

**Hemin synthesis with glycine containing  $C^{14}$  in its alpha-carbon atom.** K. I. AITMAN, G. W. CASARETT, R. E. MASTERS, T. R. NOONAN, and K. SALOMON, (introduced by W. F. BALE) *University of Rochester School of Medicine and Dentistry, Atomic Energy Project, Rochester 7, New York*. It has been shown by Shemin and Rittenberg (*J. Biol. Chem.* 159, 567, (1945), 166-621, (1946)) that the nitrogen component of glycine is incorporated in the hemin of hemoglobin. This left unanswered the question whether the carbon skeleton of glycine would also function as a specific precursor of the pyrrole in hemin. In order to investigate the latter point,  $C^{14}H_2NH_2COOH$ , prepared by B. M. Tolbert (Radiation Laboratory, University of California), was fed to rats which had been rendered anemic by previous phenylhydrazine feeding. These animals were rapidly forming new erythrocytes as indicated by a high reticulocyte count. The radioactive gly-

cine was administered by stomach tube in a single dose of approximately 1  $\mu$ c/100 gm. body weight. At appropriate intervals animals were bled as completely as possible from the carotid artery. Hemin was prepared following Nencki, M. and Jaleski, J. (*Ztschr. f. physiol. Chem.* 30, 384, (1900)), globin according to Anson, M. L. and Mirsky, A. E. (*J. Gen. Physiol.* 13, 469, (1930)), and hemoglobin by crystallization after hemolysis.  $C^{14}$  was determined according to a method developed by W. F. Bale and R. E. Masters. In a typical experiment the concentration of  $C^{14}$  per gram hemin was 9.65 times that found per gram globin. From this evidence we conclude that the alpha carbon of glycine is a direct precursor of a portion of the hemin molecule.

**Extraction of myogen and myosin from whole skeletal muscles.** WILLIAM R. ANDERSON, ROBERT S. ANDERSON (by invitation), BETTY CHINN (by invitation) and T. J. IRVING (by invitation) *Department of Physiology, University of Maryland, Baltimore, and Institute of Physical Chemistry, University of Uppsala, Uppsala*. Myogen and myosin may be partially extracted from whole skeletal muscles (rabbit) without munging the tissues. Whole muscles are placed in closed bottles and quickly frozen in  $CO_2$  snow. After they thaw the extracting solutions are poured over them (1 to 2 cc. per gram). Extraction is rapid in the first day, smaller increments of protein continue to appear for many days. Extraction proceeds best at 0°C. After several days the solutions are poured off, centrifuged, and dialyzed against phosphate buffers. Protein solutions thus prepared show the following characteristics: (1) Distilled water extracts contain myogen up to 2% of the wet weight. In extracts of white muscle the electrophoretic patterns, in phosphate buffer at pH-7.6, show a large, slow component, and a small, fast one. In red muscle extracts the same components occur, plus a myoglobin elevation, intermediate in velocity. These extracts contain no myosin, and have a low viscosity. (2) 0.4M K phosphate buffer at pH-7.6 extracts more protein, but the electrophoretic patterns are still simple, consisting of the myogen components just described. No myosin appears for many days. (3) If  $Na_2P_2O_7$  (500 mgm. %) is added to the phosphate buffer, myosin is extracted from whole muscles, both red and white, so that the solutions become very viscous. The pyrophosphate also increases the extraction of the myogen components. Sharp myosin elevations appear in both the electrophoretic and ultracentrifuge sedimentation diagrams. Actomyosin and actin are not extracted. The myosin may be separated from the other proteins by electrophoretic fractionation.

**A study of various methods of rewarming men after exposure to extreme cold.** ADELBERT AMES, III (by invitation), RICHARD S. GRIFFITH (by invitation), DAVID A. GOLDTHWAIT (by invitation), MARTIN B. MACHT (by invitation), and H. S.

**BELDING** *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass* To determine the efficiency of various methods of rewarming individuals after exposure to extreme cold, oxygen consumption, skin temperatures, rectal temperatures, thermal comfort and onset of shivering of four men were determined during 15 experiments, each consisting of a one hour exposure to  $-40^{\circ}\text{F}$ , followed by one hour of rewarming and one hour of re exposure to  $-40^{\circ}\text{F}$ . Rewarming was attempted by removal of subject to higher ambient temperature, i.e.,  $40^{\circ}\text{F}$  or  $80^{\circ}\text{F}$ , moderate or strenuous exercise, i.e., walking on treadmill 2.5 mph at level or 3.5 mph at 6.5% grade, providing additional thermal insulation (heavy sleeping bag), infrared irradiation of hands or face. The order of efficiency of various rewarming methods, as measured by change in total body heat, was (1) strenuous exercise, (2) ambient temperature of  $90^{\circ}\text{F}$ , (3) sleeping bag, (4) ambient temperature of  $40^{\circ}\text{F}$ , (5) moderate exercise, (6) irradiation of face, (7) irradiation of hands. The first four procedures caused increases in total body heat during the rewarming period, but only in the first was the original heat content approximated. Moderate exercise resulted in cessation of heat loss without gain during rewarming. Irradiation of hands or face had no significant effect on total heat content. In all cases, body heat decreased during the second hour of cold exposure at approximately the same rate as during the first hour. The only indications of acclimatization, based on results of control experiments, were a progressive increase in thermal comfort and a progressive delay in onset of shivering during successive exposures.

**Relation between synaptic delays and characteristics of preganglionic volleys initiating responses in cat autonomic ganglia** DOMINGO AMPL (by invitation) and HARRY GRUNDFEST *Dept of Neurology, College of Physicians and Surgeons, Columbia Univ, New York*. Synaptic delays in the superior cervical, stellate, and inferior mesenteric ganglia of the cat have been determined in relation to the type of initiating preganglionic impulse, which may be either of the A or the B fiber type. Both fiber types were found in the preganglionic nerves to the 3 ganglia, but their relative importance varied in different preparations. Six successful preparations of each ganglion, with minimally damaged circulation were studied. Two or all 3 ganglion preparations were made in 4 animals.

Afferent activity was produced by graded electrical stimulation of the preganglionic nerves. Simultaneous oscillographic records of the electrical activity of the preganglionic and postganglionic nerves and of the ganglion permitted correlation of the time relations of the ganglionic response with the conduction velocity of the initiating preganglionic volley. Because of the known relation between fiber velocity and spike duration these

experiments afforded a test of the relative importance of the duration of the afferent spike in determining synaptic delay.

Preganglionic volleys beginning with fibers conducting at 34.8 to 17.5 mps, therefore of the A type, with spike durations approximately 0.5 msec, produced ganglionic responses after synaptic delays of 1.9 to 4.1 msec. Preganglionic activity if predominantly B group fibers (volleys with highest velocities of 15.8 to 6.2 msec, spike durations about 1.2 msec) produced ganglionic responses after delays of 2.5 to 5.0 msec. Therefore, the nature and duration of the initiating spike apparently are not the primary determinants of synaptic delay in autonomic ganglia.

**Changes in nitrogen distribution in the Japanese beetle during metamorphosis.** JOHN M. ANDERSON (introduced by OTTO M. HELFF) *Dept of Biology, Brown Univ, Providence, R I*. Lacking histological data, an attempt was made to trace by chemical methods the processes of histolysis and histogenesis during metamorphosis of the Japanese beetle. Analyses of individual insects at all metamorphic stages yielded data for (1) insoluble protein and chitin N, (2) amino acid N, (3) soluble protein, proteose and peptone N, and (4) uric acid N. These values were expressed in percent of total N at each stage and their fluctuations charted. A conspicuous change occurs at pupation. Fraction (1) decreases, amino acid N and Fraction (3) suddenly increasing. Changes in the reverse direction have occurred by the 3rd pupal day, and late-prepupal levels are then gradually reconstituted. Uric acid N reaches its peak by the 3rd pupal day and apparently shows no further changes. No differences were found between late pupae and adults.

It is assumed that decrease in insoluble N and increase in soluble N indicates histolysis, reverse changes indicating histogenesis. The reciprocal shifts in these fractions at pupation do not accompany, but follow, the fall in oxygen consumption and in blood pH found by Ludwig (1931, 1934). These latter changes may be considered as manifestations of processes occurring prior to disintegration of larval tissues at pupation.

**Chloruretic action of pressor-antidiuretic fraction of posterior pituitary extract** W. PARKER ANSLOW, JR., LAURENCE G. WESSON, JR., ALFRED A. BOLOVER and JOHN G. TAYLOR (introduced by HOMER W. SMITH) *New York University College of Medicine*. The chloruretic action of the pressor-antidiuretic fraction of posterior pituitary extract (Pitressin) has been examined under conditions which preclude contribution to chloruresis by the oxytocic principle (Pitocin) and by changes in filtration rate and plasma sodium and chloride. In 5 of 6 experiments on 2 dogs under water load (presumably minimal endogenous posterior pituitary secretion) and under conditions otherwise conducive to

stable chloride excretion, the rate of excretion on discontinuing Pitressin infusion fell to a level one half or less the maximal rate during Pitressin administration (15-20 m $\mu$  per hour). Changes in sodium and potassium excretion generally paralleled changes in chloride excretion. Ammonium excretion was unchanged. In experiments with no water load (presumably minimal endogenous posterior pituitary secretion), no significant change in chloruresis was observed when Pitressin was administered or discontinued. In two experiments the action of Pitressin was compared with that of Pitocin at a dose of the latter which gave little or no antidiuretic effect (26 m $\mu$  of Pitocin per hour). No chloruretic effect was observed. The results indicate that the pressor-antidiuretic fraction is chloruretic, but that this chloruretic action is demonstrable experimentally only when endogenous secretion is minimal (i.e., during water load). No new evidence is adduced as to whether the chloruretic action is attributable to the antidiuretic hormone or not.

**Physiological significance of preserved central vision in lesions of the optic tract vs. optic radiation.** GEORGE M. AUSTIN (by invitation), F. H. LEWIS and FRANCIS C. GRANT (by invitation). *Lab. of the Neurosurg. Service, Hosp. of the Univ. of Pennsylvania.* The present theory of cortical representation of the macula postulates macular sparing in the visual field with lesions of the occipital lobe, macular splitting with lesions of the optic tract. It is also believed that a lesion large enough to involve the entire optic radiation or area 17 (striata), will produce hemianopia with macular splitting. Contrariwise, clinical evidence has suggested a bilateral macular representation in the occipital lobes. We have examined persons with complete occipital lobectomy including the entire area striata. Central visual fields were mapped out with the Bjerrum tangent screen and the Lloyd stereocampimeter. These patients showed only a small area of preserved central vision varying from  $\frac{1}{4}$  to  $1\frac{1}{2}^{\circ}$ . Central field studies of patients with optic tract lesions revealed similar small areas of preserved central vision. The macula includes an arc of approximately  $10^{\circ}$ C, the fovea an arc of approximately  $2^{\circ}$ . Our observations failed to show macular splitting in optic tract lesions or macular sparing following interruption of the optic radiation, or removal of the striate cortex. The absence of foveal sparing vindicates against a bilateral macular cortical representation, for this presupposes the preservation of at least an arc of the fovea. The absence of hemianopia with macular splitting after complete removal of area 17 or the optic radiation may be explained by the use of a slightly eccentric foveal point for fixation.

**The role of the bulbar facilitatory and inhibitory systems in vasomotor and respiratory activity**

L. M. N. BACH (introduced by H. S. MAYERSON). *Department of Physiology, Tulane University School of Medicine, New Orleans.* The reticular matter of the brainstem has been shown to play a most important part in facilitation and inhibition of reflexly and cortically induced somatic motor activity. Comparison of points in the reticular system producing these effects with those found controlling vasomotor and respiratory activity shows a high degree of coincidence. Simultaneous records were made of the knee-jerk, blood pressure and respiration in the cat while selected points in the reticular matter of the pons and medulla were stimulated. It was found that stimulation of points which caused complete inhibition of knee-jerk always caused a marked drop in blood pressure as well as a total inhibition of all respiratory movements usually persisting for the duration of the stimulus. Following cessation of the inhibitory stimulus, whenever there was a rebound effect on the knee jerk (post-inhibitory facilitation) there also occurred simultaneously a marked increase in blood pressure and in both rate and depth of respiration. These effects occurred simultaneously for equal periods of time, declining similarly to the normal values. Converse vasomotor and respiratory effects were found in knee jerk facilitation with post-facilitatory inhibition.

Apparently bulbar facilitatory and inhibitory systems apply equally to vasomotor, respiratory, and somatic reflexes. Since it was impossible to obtain pure vasomotor or respiratory effects without definitely affecting knee-jerk activity, it may be assumed that no completely specific brainstem vasomotor or respiratory centers exist. To varying degrees the reticular matter in these areas seems simply to facilitate or inhibit spinal motor cells in general.

**Peripheral vascular effects produced by localized warming of various skin areas.** MORTIMER E. BADER, MARTIN B. MACHT, and ELIZABETH L. PILLION (introduced by H. S. BELDING). *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Massachusetts.* By means of a venous occlusion plethysmograph and thermocouples placed on selected skin areas, the effect on blood flow through the hand produced by warming localized body areas was studied. A 250 watt infrared bulb located at the base of an insulated metal cylinder (diam. 20 cm.) and controlled through a rheostat was used as a heat source. The subject was seated comfortably with his left hand in an air plethysmograph at heart level and the open end of the cylinder was applied to the area being warmed, a diaphragm being used to prevent heat leakage. Rectal temperature, hand skin temperature, and skin temperature at five widely separated points were determined during each experiment. Subjects remained inactive at an environmental temperature of  $15^{\circ}$ C until hand

skin temperature reached equilibrium (approximately 17°C) at which time basal blood flows and skin temperatures were measured.

Using two subjects in repeated experiments it was demonstrated that heating the face to 44–45°C resulted in significant increases in hand skin temperature (average rise 10.2°C) and average maximum flows 4 times as great as the basal values. Increases generally became apparent within one hour and continued until termination of the experiment at 90 minutes. No changes in rectal temperature, or skin temperature elsewhere were observed. Control experiments, applying heat to the chest in identical fashion, produced no significant changes in hand skin temperature or blood flow. These results suggest that warming certain skin areas is more effective than warming others in overcoming the vasoconstriction of the extremities produced by cold.

**Hepato-renal factors in circulatory homeostasis.** XX Antidiuretic action of liver VDM concentrates. S. BAEZ (by invitation), A. MAZUR and EPHRAIM SHORR. *Dept. of Medicine, Cornell University Medical College and The New York Hospital, New York City.* Extracts of anaerobic liver chemically fractionated to concentrate their VDM component were found to produce a profound antidiuretic effect upon intravenous injection into unanesthetized dogs. The VDM content of the liver concentrates, as assayed by the rat mesoappendix technique, was such that 0.001 to 0.0001 gamma N produced a well defined vaso depressor effect in the test rat. The amount of VDM infused into the dog for antidiuretic study varied from about 50 to 200 gamma N per minute, depending on the potency of the particular preparation. The rate of injection was regulated so as to maintain a relatively uniform blood level of VDM sufficient to give a transferable vaso depressor effect in the test rat. The infusion was continued for 60 to 90 minutes, a total of 2.0–20.0 mg VDM N being administered in the different experiments. The amounts injected were for beef liver VDM 7.0–12.0 mg N, for horse liver 2.5–3.0 mg N, and for dog liver preparations 2.0–2.5 mg of VDM N. In one series of experiments a study was made of the effect of VDM in the diuresis produced by the infusion of 5% dextrose. In a second series the effects on water diuresis (500 cc by stomach tube), were determined. The infusion of the VDM preparations resulted, within 10 to 15 minutes, in a 70–80% reduction in urine flow which was sustained throughout the period of infusion. The relation of the antidiuretic effect to the VDM content of the preparations and to possible extraneous materials will be discussed.

**A Geiger-Muller counter system for tracer studies of gas exchanges in man.** A. C. BARGER (by invitation), G. S. RICHARDSON (by invitation) and E. M. LANDIS. *From the Department of Physiology, Harvard Medical School.* An atmospheric pressure

Geiger-Muller counter (Brown, Good and Evans) has been modified for the purpose of determining the kinetics of gas exchange in lower animals and man. The counter, consisting of an oxidized copper tube and tungsten center wire, has been inserted directly into a closed respiratory circuit. This permits rapid recording of saturation or desaturation with minute amounts of a wide variety of radioactive gases, including A<sup>37</sup>, the radiation from which is so weak that it is detectable only when introduced into the sensitive volume of the counter itself. An automatic feed-back compensator is incorporated into the system to correct for limited changes in gas composition. A mixture of 80% helium with 20% oxygen has proved to be a much more suitable filling gas than air or oxygen because it requires lower voltage and yields lower background with better reproducibility.

Curves showing the absorption of A<sup>37</sup> in man may be roughly divided, for preliminary analysis, into three parts — (a) a rapid decline in counting rate lasting from 1–2 minutes, representing chiefly pulmonary mixing, (b) a much less rapid decline lasting from 4–7 minutes during which argon enters the blood and presumably the more richly vascular tissues, (c) a very slow decline representing more gradual penetration of other regions of the body.

**Determination of protein-bound iodine in biological material.** S. B. BARKER and H. J. LIPNER (by invitation). *Department of Physiology, State University of Iowa, Iowa City.* A procedure has been evolved, by combining and modifying previous methods, for the determination of protein-bound iodine in various tissues, including blood plasma. The principal steps are precipitation, washing and oxidation of the protein, distillation of the iodine into sulfite, elimination of SO<sub>2</sub> following acidification, and colorimetric determination of the iodide by means of its catalytic effect on the reduction of ceric ions by arsenious acid. Advantage is taken in the last step of the chloride enhancement of iodide catalysis pointed out by Sandell and Koltzoff. This method permits a satisfactory analysis to be performed on 2 ml. of plasma, one-fiftieth of a normal rat thyroid gland, or 500 mg. of rat liver, kidney, heart or skeletal muscle. The changes in protein-bound iodine produced by injection of thyroxin and of elemental iodine are being studied by means of this procedure.

**Inulin, urea, mannitol, and PAH clearance ratios in premature infants.** HENRY L. BARNETT (by invitation), HELEN McNAMARA (by invitation), RUTH S. HARE (by invitation) and KENNEDRICK HARE. *New York Hospital and the Department of Pediatrics, Cornell University Medical College, New York City.* Simultaneous measurements of 98 inulin and urea clearances and 69 inulin, urea, and mannitol clearances were made in 10 premature infants ranging in weight from 2156 to 2470 Gm.



(surface area from 161 to 182 sq M) The inulin U/P ratios ranged from 3.9 to 127 and exhibited a close inverse relationship to the urea/inulin clearance ratios which ranged from 0.25 to 0.95. The average mannitol/inulin clearance ratio was  $0.90 \pm 0.07$ . In 8 of the premature infants whose ages ranged from 9 to 21 days, the average inulin and mannitol clearances were  $4.37 \pm 0.88$  and  $3.93 \pm 0.60$  ml/min, respectively. In contrast to the findings of Berger, et al. in adult subjects (Proc Soc Exper Biol & Med 66:62, 1947), the inulin clearances were not lower after mannitol in 5 infants whose control inulin clearances were measured prior to the infusion of mannitol. PAH clearances with plasma levels less than 2.0 mg/100 ml and PAH tubular maxima with levels greater than 50 mg/100 ml were measured in 4 of the above infants who were less than 14 days old. The average PAH clearances and tubular maxima were 13.2 ml/min and 0.97 mg/min, respectively. If PAH clearances at plasma levels below 2.0 mg/100 ml measure renal plasma flow in these infants, the average filtration fraction calculated as the inulin/PAH ratio is 0.34. Higher values for all of these measurements in infants of the same weight range (2 to 2.5 Kg) but over 50 days of age suggest that development of kidney function correlates more closely with postnatal than with gestational age.

#### CO<sub>2</sub> content of maternal and fetal sheep blood

DONALD H. BARRON *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn.* Observations of the CO<sub>2</sub> content of blood in the uterine and umbilical vessels in pregnant sheep in the last half of the gestation period indicate: 1) that the content of the fetal blood is higher than that of the maternal, 2) that the content of the fetal blood tends to increase regularly as gestation advances, whereas 3) no such uniform change has been observed in the CO<sub>2</sub> content of the maternal blood, 4) the CO<sub>2</sub> content of the blood in the umbilical artery tends to be uniformly higher by four or five volumes per cent than blood in the umbilical vein.

**Molecular structure and activity of "vitamin P"-like substances. Inhibition of succinoxidase.** GRANT R. BARTLETT (introduced by WILLIAM G. CLARK) *Scripps Metabolic Clinic, La Jolla, Calif.*

Elsewhere (Clark and Geissman, Fed Proc, 1948) it was pointed out that "vitamin P"-like compounds with an ortho-dihydroxyphenolic structure, a side chain containing an unsaturation and a carbonyl, are epinephrine potentiators (antioxidants?).

In considering possible tissue reactions of these substances, it is recalled that the ortho-diphenols are readily oxidized to quinones which in turn could be toxic to enzyme systems requiring —SH groups for their activity. The compound 2',3,4-trihydroxychalcone was found by Clark and Geissman to be one of the most active epinephrine antioxi-

dants. It gave a strong inhibition of the —SH enzyme succinoxidase, the inhibition increasing at pH values above 7. At pH 7.4,  $10^{-4}$  M chalcone almost completely inhibited the enzyme. The cytochrome C-cytochrome oxidase component of the enzyme system was not affected. Glutathione prevented but did not reverse the inhibition. When one of the ortho dihydroxy groups is covered as in 4-methoxy, 2',3-dihydroxy-chalcone, a quinoid form could not be obtained on oxidation, and succinoxidase is not affected. In the light of the known reactions of quinones, the evidence favours the view that the enzyme inhibition is due to a quinoid oxidation product.

**Relationship between arterial pressure and renal blood flow.** WOODROW BATTEN (by invitation), BEN C. OCHS (by invitation), CARLOS RAFFELA (by invitation—Rockefeller Fellow), J. ROY HIGGINS, JR. (by invitation), J. MAXWELL LITTLE, HAROLD D. GREEN. *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* A kidney and the distal stumps of the aorta and vena cava were progressively dissected free from a dog (kidney dog) until they were connected only by the proximal portion of the aorta and vena cava. A donor dog was heparinized and its femoral artery connected with the distal stump of the kidney dog's aorta. The distal stump of the kidney dog's vena cava was connected with an outflow meter. The proximal stumps of the kidney dog's aorta and vena cava were then ligated and severed thus completing the isolation of the kidney without its being at any time subjected to ischemia. The perfusion pressure was controlled by an air chamber and automatic electric clamp on the femoral artery of the donor dog. The pressure was lowered for periods of one-half to fifteen minutes to a given level and then returned to the control pressure. Control flows averaged 230 ml/min/100 gm of kidney (range 115 to 450) at arterio-venous differences of pressure of 120 mm Hg. Satisfactory plots of flow vs. A-V difference of pressure were obtained in nine experiments. Of these only one gave a curve concave to the pressure axis as described by Selkurt. Two were convex and the remainder were straight. All tended to intercept zero flow at approximately 20 mm Hg pressure. In this isolated kidney preparation no evidence of autoregulation of flow was observed.

Preliminary studies suggest that a permanent decrease in PAH clearances occurred early in the course of these experiments. The cause of this is being investigated.

**A further study of the effect of hemoglobin on cellular respiration.** I. PERCY BAUMBERGER, GEORGE F. LEONG (by invitation) and KATHLEEN BARDWELL (by invitation). *Physiology Depart-*

ment, *School of Medicine, Stanford University* Yeast cells increase their oxygen consumption rate at low oxygen tensions when oxyhemoglobin and hemoglobin are added to the suspension. Several working hypotheses were tested in seeking an explanation of this phenomenon which has been studied by Neumann (1935), Lmodi and Sarkany (1937) and Brumberger (1938). The hypotheses may be stated as follows—*The increase in  $Q_0$  of yeast on the addition of oxyhemoglobin-hemoglobin is due to*—(1) an improved oxygen supply to the yeast cells because of the liberation of oxygen from the oxyhemoglobin, or (2) the catalytic action of the methemoglobin that may be formed, or (3) the liberation of some component of the erythrocyte, other than hemoglobin, or (4) the hemoglobin itself. Spectrophotometric and polarographic methods were employed in testing out these hypotheses. The first three are untenable but the fourth hypothesis has merit. So far it can be said that oxyhemoglobin-hemoglobin increases the  $Q_0$  of yeast whether it is in the intact erythrocyte or freed by hemolysis or is purified but it has no effect if oxidized to methemoglobin or combined with carbon monoxide. The percentage increase in  $Q_0$  is a linear function of the log of the percentage hemoglobin in the oxyhemoglobin-hemoglobin mixture.

The femoral A-V glucose differences following glucose infusion in unanesthetized normal and adrenalectomized dogs. C. H. BEATTY (Introduced by MAGNUS I. GREGERSEN) *Department of Physiology, College of Physicians and Surgeons, Columbia University*. It is generally agreed that the disappearance rate of injected glucose in adrenalectomized animals in good condition is at least as rapid as in normal animals. However, it is not clear whether the sites of disappearance are identical. In the following experiments the femoral A-V glucose differences were measured in 6 control and 4 bilaterally adrenalectomized dogs in good condition. Glucose (0.75 gm./kgm.) was injected intravenously in 10 cc. of water and blood samples were taken at 15, 30, 45 and 60 minutes. A statistical analysis of the femoral A-V glucose differences following glucose infusion showed that these were the same in normal and adrenalectomized animals. The fact that no significant variations in blood flow were found after glucose injection in 2 heparinized control dogs ( $\pm 10\%$  by bleed out method) indicates that glucose itself produces no changes in the rate of blood flow. But since no blood flow measurements were made upon the adrenalectomized dogs this evidence on the rate of glucose utilization is incomplete.

Oxyhemograph studies during marked vasomotor changes. VIVIAN G. BEHRMANN (introduced by ROBERT GESELL) *Department of Laboratories, Henry Ford Hospital, Detroit, Michigan*. The photoelectric oxyhemograph recently described by

Hartman, Behrmann, and Chapman is so constructed that vasomotor changes are cancelled out in the continuous recording of  $O_2$  saturation under ordinary circumstances. No evidence of vasomotor disturbances has been observed in a series of anesthesia studies on experimental and clinical subjects. However, fluctuations in the  $O_2$  saturation curve have been induced in the experimental animal on the administration of vasodilator (histamine) and vasoconstrictor (adrenalin) drugs, in amounts sufficient to create marked blood pressure alterations. This prompted a search for the factors responsible for these variations. Blood volume changes at the site of the photocell as well as analyses of the blood draining this area should offer valuable information in the solution of this problem. Therefore, all veins, except the main trunk of the anterior auricular vein, were tied off at the base of the ear a day or two previous to the experiment. Blood volume flow was registered from the cannulated anterior auricular vein through a Gibbs drop recorder. The blood, draining the ear photocell site, was collected under oil and analyzed for comparison with the  $O_2$  saturation values obtained on arterial blood by Van Slyke's manometric method. The effects of various vasoconstrictor and vasodilator agents on the blood oxygen saturation curve, coincident with tracings of mean blood pressure, pulmonary ventilation, and blood volume flow from the photocell site are shown.

The tolerance of dogs to intravenously administered fatty chyle and synthetic fat emulsion. ISAAC MURPHY BERRY (by invitation) and A. C. ILLI *Dept. of Clinical Science, University of Illinois College of Medicine, Chicago*. Doses of one gram of fatty acids per kilogram of body weight were given 10 fasting dogs intravenously over a period of about 12 minutes in the form of fatty chyle collected from cannulated thoracic ducts of donor dogs, and in the form of the 10% butter oil emulsion prepared according to the method of Freeman and Meng (average particle size less than one micron). The average level of blood fatty acids for the 10 dogs, expressed as milligrams percent of oleic acid at 0, 0.5, 1, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5 hours after injection of the synthetic fat emulsion was 204, 450, 437, 410, 365, 362, 324, 295, 295, 277, and 232 compared to 212, 537, 365, 313, 212, 202, 206, 211, 217, and 186 after the injection of the same amount of fat as chyle. The disappearance from the blood stream of the injected fat was significantly more rapid when the fat was in the form of chyle than when it was in the form of the synthetic emulsion.

Cerebral cortical effects of curare. RICHARD G. BERRY, LIEUT. (MC) US Navy, and FRANCIS M. FORSTER (Introduced by M. H. F. Friedman) *Dept. of Neurology, Jefferson Medical College, Philadelphia*. The application of curare to the ex-

posed cerebral cortex of cats, previously anesthetized with Dial, results in the appearance of increased electrical activity of the cortex within four to six minutes. This increased activity is manifested in the electrocorticogram by diphasic spikes similar to those obtained with strychnine, acetylcholine, metrazol and picrotoxin. When the curare is applied to area 7, these spikes show typical transneuronal firing to the contralateral area 7, and when applied to the acoustic cortex it is possible to "drive" the spikes by appropriate stimuli in an analogous manner to the acoustic driving of strychnine and acetylcholine discharges. There is no preliminary cortical depression as noted with ACh. When applied to the motor cortex only minimal and inconstant skeletal muscle response is noted. Varying the pH of the solution did not alter the discharges produced by *Intocostrin* (Squibb) and d-tubocurarine chloride. It is concluded that curare, applied directly to the cerebral cortex of cats, has a stimulating effect on the electrical activity of the cortex.

A new concept of phase-boundary potential applied to the electro-physiology of nerve. R. BEUTNER and T. C. BARNES, *Dept. of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia*. The rapidity of the rise of electrical potential produced by an alkaloid added to the phase boundary between an oil layer and an aqueous medium suggests that the spike potential in living nerve may be a phase boundary potential (Barnes and Beutner, *Science* 101: 569, 1946; Barnes, *Fed. Proc.* 6: 73, 1947; Seventeenth International Physiol. Congress 390, 1947). By the courtesy of Dr. H. K. Hartline cathode ray oscillograph records were obtained with a DC amplifier used for electroretinograms. The addition of 0.07 cc of 0.0027 M acetylcholine chloride in saline to a guaiacol interface in 200 cc of saline produced a wave of negativity of 2 millivolts lasting 0.2 of a second. This wave of negativity which resembles the action current in living nerve was followed by small positive and negative components not unlike after-potentials. Like the spike potential in nerve the acetylcholine potential is reversible as indicated by the return of the wave to the base line and by the following conductivity measurements. 20 cc of guaiacol was shaken for two hours with 100 cc of 0.0027 M acetylcholine chloride in saline and the electrical resistance of the oil was  $5.75 \times 10^5$  ohms compared with  $> 2.5 \times 10^6$  ohms for guaiacol control shaken with saline. The oil containing the acetylcholine was then reshaken with saline and the resistance returned to the original value of  $> 2.5 \times 10^6$  ohms. These investigations afford additional evidence disproving the old membrane theory of bioelectrical potential in nerve.

Survival of adrenalectomized-nephrectomized rats treated with desoxycorticosterone. JAMES H.

BIRN (by invitation), W. J. EVANS (by invitation) and ROBERT GAUNT, *Department of Zoology, Syracuse University, Syracuse, N. Y.* Studies on the extra-renal action of desoxy corticosterone acetate (DCA) in water intoxication (in press) led to observations on the effect of this steroid on the survival of nephrectomized animals, also marked differences in the survival of rats of different ages after nephrectomy were observed. In male rats weighing approximately 130 grams, the right adrenal and kidney were removed in one operation and the left adrenal and kidney removed one week later. One group was given 3 mgm of DCA per day beginning immediately after the second operation. A second group received 0.5 cc of peanut oil in a similar fashion. In a third group the kidneys but not the adrenals were removed. No treatment was given any group prior to nephrectomy.

Adrenalectomized nephrectomized rats treated with DCA survived on the average 51.1 hours (9 animals). Adrenalectomized nephrectomized rats given peanut oil survived 30.3 hours (10 animals). Rats which had been nephrectomized only survived 29 hours (20 animals). These results indicate that, for the size of rat used, DCA exerts a protective action against the effects of nephrectomy which is greater than that offered by the intact adrenal gland.

In rats that were nephrectomized and untreated those weighing 81 to 120 grams survived 27 hours (12 animals), those between 210 and 322 grams survived 61 hours (8 animals), and those in between these extremes showed an intermediate survival time (11 animals). These results explain some of the wide discrepancies in the literature concerning survival times of nephrectomized rats.

The recovery of sodium thiocyanate from whole blood and plasma as related to the measurement of extracellular fluid volumes. E. W. BIRBY (introduced by H. C. BAZETT), *Physiology Department, Medical School, University of Pennsylvania, Philadelphia, Pennsylvania*. It has been found that extracellular fluid volumes, using the sodium thiocyanate method, increased in malarious humans with the severity of the infection (Overman, Davis, and Thorpe, *Amer. Physiol. Soc. Fed. Proc.* 6: 1, pt 2, 174, 1947). In order to study the permeability of the malarious erythrocyte to thiocyanate, normal whole blood has been used as a preliminary control. Whole blood and plasma were studied separately in their reactions to both concentrated and dilute solutions of sodium thiocyanate. When the concentrated solution of thiocyanate (4.71%) was mixed with blood and plasma, the final concentration of thiocyanate was 13.5 mgm/cc. With plasma all the thiocyanate was recoverable within the limits of accuracy (1-2%). With whole blood, equilibrium between erythrocytes and thiocyanate

was found when the thiocyanate concentrations of the separated erythrocytes and plasma were analyzed separately. Dilute thiocyanate 0.1% gave a resultant concentration of 0.5 to 0.25 mgm per cc in plasma and blood. These concentrations approach the usual range of thiocyanate level found in the serum of subjects on whom extracellular fluid volumes are determined by the thiocyanate method (Crandall and Anderson, *Am J Digest Dis & Nutrition*, 1: 126, 1935).

When plasma alone was used, 10-20% of the thiocyanate added could not be recovered. In these dilute concentrations, small increases of thiocyanate added to a constant plasma volume gave higher percentage values for thiocyanate recovery. And the reverse was likewise true. When whole blood was used with this dilute concentration of thiocyanate only 5-10% of it could not be recovered. If the loss of thiocyanate due to plasma alone is considered, this is evidence that equilibrium between erythrocytes and thiocyanate is established in dilute concentrations.

**A light-stable visual purple.** ALFRED F. BLISS, *Department of Physiology, Tufts College Medical School, Boston*. Squid retinas contain cephalopsin (*Biol Bull* 91: 220), a visual purple similar to that of vertebrates in its absorption spectrum and in that it can be bleached thermally or by polar solvents, yielding a lipid called indicator yellow, which in acid solution releases the petroleum ether-soluble carotenoid, retinene. However, when dark-adapted squid retinas are illuminated continuously one hour after excision into petroleum ether, they show neither bleaching nor increased retinene release in light intense enough to bleach a vertebrate retina in less than a minute. On the other hand, when squid retinas are illuminated immediately after excision into petroleum ether, the thermal rate of retinene release is doubled and maintained for more than three hours. It is concluded, from the maintenance of the light-initiated retinene release in spite of the demonstrated absence of a light sensitive retinene precursor in one hour old retinas, that there is no present evidence for a photolabile visual purple in the squid. It is suggested that the light effect is an acceleration of thermal bleaching caused by the accumulation of polar metabolic products in the stimulated retina.

**The source of uropepsin in man.** STANLEY BLOCK (by invitation), LOUIS ROSENBERG (by invitation), R. H. BROTHMAN (by invitation) and I. ARTHUR MIRSKY, *May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio*. The urine of subjects with an intact, functioning stomach contains a pepsin-like enzyme which has been designated as uropepsin. An attempt has been made to determine the site of formation of this enzyme and the mode of its entrance into the urine. Previous studies have indicated that this enzyme

originates from the stomach. This has been inferred from its absence from the urine of gastrectomized animals and of human patients with pernicious anemia. We have extended these observations by demonstrating its absence from the urine of gastrectomized patients. Although uropepsin originates from the stomach it appears to be absorbed directly into the blood stream from the secreting peptic glands rather than after its excretion into the lumen of the stomach. This was demonstrated by feeding large amounts of gastric pepsin to patients with pernicious anemia, to normal human subjects and to dogs. In no case did this procedure result in an elevation of the levels of uropepsin excretion which had existed prior to the oral administration of the enzyme. The intravenous administration of pepsin to dogs did not result in an increase of uropepsin excretion. Such an observation indicates the inability of pepsin to be excreted after its introduction into the blood and is presumptive evidence that the enzyme is transported intravascularly in the form of pepsinogen, a finding which also would tend to confirm the hypothesis that the enzyme is absorbed directly from the secreting cell in the form of its inactive precursor, pepsinogen.

**A method for production of cardiac infarction in the dog.** J. RICHARD R. BOBB, DONALD C. KUNZE and WM. McCALL, JR. (Introduced by HAROLD D. GREEN), *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina*. Simple ligation of a coronary artery or a coronary artery and its accompanying vein has given irregular unpredictable areas of necrotic myocardium and at times no necrotic myocardium could be found at all. Since the major vessels of the heart are on the surface it was felt that possibly the collateral circulation which supplies the myocardium after ligation of its primary supplying artery has its main branches, although very small, also on the surface. To test this idea dogs were anesthetized with intravenous sodium pentobarbital (40 mg/kg), artificial respiration was administered and aseptic thoracotomies performed. Ligation of the anterior descending arteries and accompanying veins was done in ten dogs. The arborizations of the ligated vessels were exposed and a cautery touched lightly around the area supplied by the ligated artery. The chests were closed aseptically. All dogs showed definite gross infarcts when sacrificed about ten days postoperatively. In eight control animals the anterior descending arteries and veins were ligated similarly to the test animals. No cauterization was done. Two of these showed extensive infarcts. Four showed infarctions of varying size and two showed no evidence of infarction. Four controls were done in which the only cardiac lesion was a superficial burn around the myocardium supply-

by the anterior descending artery. None of these animals showed infarction. For three days post-operatively a total of 100,000 units of penicillin was administered in divided doses. In a series of 25 dogs with thoracotomies there were no deaths from infection.

It is believed this method will produce a cardiac infarction with greater consistency and of more predictable size than simple ligation.

**Change in riboflavin during embryonic development.** J. H. BODINE and L. R. FITZGERALD (by invitation). *Zoological Laboratory, State University of Iowa.* A quantitative study has been made of the rate at which riboflavin is converted into pterines during the course of embryonic development of the grasshopper. By the use of  $\gamma$ -irradiation it has been possible to destroy parts of the egg and to determine the extent to which embryonic versus non-embryonic cells are responsible for such chemical conversions. In general it has been found that the non-embryonic cells (serosa, etc.) do not change riboflavin into pterines. Such conversions appear to be properties of the embryonic cell *per se*. A chronological table of these conversions has been worked out.

**Renal fraction in normal subjects and in subjects with essential hypertension.** ALFRED A. BOLOMEY, ERNEST F. BREED, ALEXANDER MICHIE, KATHARINE MICHIE and HENRI D. LAWSON (introduced by H. W. SMITH). *New York University College of Medicine.* Simultaneous determinations of cardiac output (C.O.) by right heart catheterization and renal blood flow (R.B.F.) by the p-aminohippurate clearance method were made on 18 non-hypertensive and 18 hypertensive subjects. The control renal fraction ( $RBF/CO \times 100$ ) averages 15.7 per cent (10.2-26.9 per cent), a value lower than the 19-20 per cent obtained when the average normal resting cardiac output reported by Cournand or by Stead is divided into the average normal value for renal blood flow reported by Smith. This discrepancy may be due to differences in the hematocrit or to hypermetabolism induced by the complexities of the procedures. C.O. showed no correlation with anemia but was increased in relation to the degree of hypermetabolism. R.B.F. showed no correlation with hypermetabolism but was decreased by anemia. Deviations in metabolic rate and in hematocrit in the hypertensive subjects were of the same order of magnitude as in the controls. The increase in C.O. associated with hypermetabolism and the decrease in R.B.F. associated with anemia operate to lower the renal fraction in both groups. The average C.O. is the same in normal and hypertensive subjects, whereas the R.B.F. varies from normal to very low values in severe hypertension, the renal fraction decreasing

to as low as 1.4 per cent in the latter (range 1.4 to 20.7, avg. 11 per cent).

**Quantitative glycogen determinations on specimens of human livers obtained by needle biopsy.** PHILIP K. BONDY, WAITER H. SHELDON and LILLIAN EVANS (introduced by JAMES V. WARREN). *Departments of Medicine and Pathology, Emory University School of Medicine.* Studies of the liver glycogen concentration in animals have produced valuable information concerning the metabolism of carbohydrates. Comparable studies in human beings have not been carried out, except under distinctly unphysiologic circumstances. Single or serial specimens of human liver tissue can now be obtained by needle biopsy under relatively atraumatic conditions. The histochemical demonstration of glycogen has been improved by Gomori's new method. A quantitative estimation of the liver glycogen content has been made by comparing the optical density of standard sections stained under standard conditions with the optical density of similar sections taken from livers, the glycogen content of which has been determined by chemical methods. Our results suggest that this method may be relied on to give results accurate to the nearest 0.5% glycogen.

Because of the irregular distribution of glycogen in the livers of animals, the results obtained by this technique can be considered only as indications of general trends, rather than as accurate measurements of the average liver glycogen content. The validity of the observations, however, is indicated by the consistency with which subjects reacted to similar influences. Furthermore, the magnitude of the changes observed was greater than the maximum range of spontaneous variation of distribution.

Normal subjects and patients with various types of disease have been studied. In cases of well controlled diabetes mellitus the liver glycogen was found to be within normal limits. At the onset of diabetic acidosis the liver glycogen was somewhat depressed. In severe acidosis the glycogen levels were reduced to less than 0.5%, but when treatment had controlled the ketosis, the glycogen levels were normal.

Overnight fasts in normal subjects produced only slight and insignificant depression of liver glycogen levels. In patients starved for periods up to 36 hours a progressive decline of liver glycogen was found. A secondary glycogen rise like that seen in experimental animals has not been observed thus far. The effect of the intravenous administration of small amounts of epinephrine has also been studied. Serial biopsies done before and after breakfast showed an appreciable increase in liver glycogen 30 minutes after eating.

Observations are also being made of the glycogen content of livers with cirrhosis and hepatitis.

**Localization of certain spasmodic respiratory**

responses in the medulla oblongata of the cat HERBERT L. BORISON (by invitation), GEORGE CLARK and S. C. WANG *Department of Physiology, College of Physicians and Surgeons, Columbia University* Systematic exploration of the medulla oblongata of the cat, by means of electrical stimulation, has shown that in addition to gross inspiratory and expiratory responses there may also be elicited a characteristic heightened spasmodic respiratory act which may be likened to such phenomena as coughing, sneezing, gasping or retching. Experiments were carried out on cats under light nembutal anesthesia or on decerebrate preparations. With the aid of the Horsley Clarke stereotaxic instrument discrete regions of the lower brain stem were stimulated with a bipolar enameled wire electrode connected to a thyratron stimulator (Rev. Scient. Inst. 18: 669, 1947). Recordings of the respiration were made with simultaneous thoracic and abdominal pneumographs or a spirometer or both. The spasmodic respiratory response could be obtained only on stimulation of the dorsolateral portion of the medulla at the level of calamus scriptorius, which corresponds to the fasciculus solitarius and its nucleus, and also to the general region of the entering glossopharyngeal and vagal rootlets. No other region of the brain stem was found to yield similar responses. Attempts will be made to determine the exact conditions which are optimal for specific expression of the spasmodic response in the various respiratory phenomena mentioned.

**The effects of anoxia upon myoglobin concentration** WILLIAM J. BOWEN and WILLIAM E. POEL (introduced by HEINZ SPECHT) *Laboratory of Physical Biology, National Institute of Health, Bethesda, Md.* Hurtado, et al. (Am. J. Med. Sci. 194: 708, 1937) reported an increase in myoglobin content of dogs born and reared at high altitudes. Their method did not differentiate between hemoglobin and myoglobin and depended upon perfusion for removal of the hemoglobin. We adopted a method based upon quantitative spectrophotometry of the carbonyl derivatives for the analysis of mixtures of myoglobin and hemoglobin which eliminates the necessity of perfusion. The muscles are cleaned, frozen with dry ice, pulverized and then simultaneously homogenized and extracted with water. The extract is cleared of interfering proteins, buffered and treated with carbon monoxide. Analysis is done at 558 and 538 m $\mu$  at which wave lengths extinction coefficients allow calculation of the concentration of myoglobin and hemoglobin. Using this method, rats, exposed 4 hours daily to 282 mm Hg (25,000 ft.) are being studied. Body weights, hematocrits and blood hemoglobins are followed as criteria of adaptation. At various stages of adaptation the rats are sacrificed and the gastrocnemius and soleus muscles analyzed. To

date, nine rats, exposed for 2 to 52 weeks, have been analyzed. In these the hematocrit and hemoglobin values had increased 25 to 66 per cent over the averages of the controls. The myoglobin, however, consistently shows no significant change from that of the controls. Also, the results show that 2 to 8 times more hemoglobin is retained by the muscles of the exposed animals than by the controls and that the amount retained varies directly with the hematocrit value.

**Alkali decomposition of myoglobin** WILLIAM J. BOWEN (introduced by HEINZ SPECHT) *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland* Among the differences reported to exist between hemoglobin and myoglobin is greater resistance of myoglobin to alkali decomposition. Haurowitz (Z. Physiol. Chem. 232: 125, 1935) gives data showing that 14 hours is required for 86% decomposition of human myoglobin by 0.5N NaOH and that 38 hours is required for complete decomposition. The same author (Z. Physiol. Chem. 183: 78, 1929) also shows that the rate of decomposition of hemoglobin by alkali varies according to species. The reactions of metmyoglobin and oxyhemoglobin of the dog and horse with N/10 NaOH have been followed with the aid of a spectrophotometer at  $24 \pm 1^\circ\text{C}$ . The extinction coefficients of 100% alkaline metmyoglobin and of oxyhemoglobin and of the respective alkali hematin at 540 m $\mu$  were used. Four experiments, using each globin in each experiment, were done. The average fractions of myoglobin (Mb) and of hemoglobin (Hb) decomposed at various intervals are given in the table. The results show that myoglobin of both the horse and dog is more resistant to alkali

| Time     | Per cent decomposed |      |      |      |
|----------|---------------------|------|------|------|
|          | Horse               |      | Dog  |      |
|          | Mb                  | Hb   | Mb   | Hb   |
| 15 sec   | 18.8                | 17.6 | 35.6 | 93.1 |
| 1 min    | 23.9                | 25.0 | 43.3 | 92.6 |
| 5 min    | 28.2                | 56.1 | 53.0 | 88.3 |
| 1 hour   | 36.8                | 94.9 | 60.8 | 87.5 |
| 24 hours | 69.7                | 95.5 | 96.5 | 93.2 |

decomposition than their respective hemoglobins. They also show that the rate of decomposition of myoglobin by alkali differs in the two species.

**Enzyme inhibitors on conduction and respiration of frog nerve** L. L. BOJARSKI, S. POSTEL, and A. ROSENBLATT (introduced by R. W. GERARD) *Dept. of Physiology, The University of Chicago* Sodium fluoracetate, 0.001M to 0.1M (over a pH range of 5 to 8) does not affect conduction or resting respiration of frog sciatic nerve (summer frogs). Methylfluoracetate, 0.005M, which should penetrate more readily, irreversibly abolished the action potential in about 3.5 hours. Extinction is faster at higher

concentrations, about 2 hours at 0.05 M. At about two hours, the action potential has been reduced 50%, but resting respiration is already inhibited by 80%. This latter differs from muscle, where only activity metabolism is affected (McKeen Cattell, Cori et al, Report OSRD 4981, June 2, 1945). Fumarate, 0.01M, applied simultaneously with, or 30 mins after, the poison, completely protects the action potential while permitting a fall of 50% in resting respiration. Acetate, alcohol, pyruvate, or succinate, at 0.01M, do not prevent the fall in action potential, though 0.05M succinate protects. Other substrates are being tested at higher concentrations, and the study is being extended to respiration of stimulated nerve. With the Gerard-Tobias micro respirometer, using 3 mg samples, the  $Q_{O_2}$  for left and right nerves agree within 10%. The coefficient of variation among all frogs (of species *Rana pipiens*) was 20% (in 32 experiments). At 30°C,  $Q_{O_2}$  during the first half-hour is  $140 \pm 28$ .

**The myenteric reflex** EMIL BOZLER *Dept of Physiology, Ohio State University, Columbus*. In the dog's small intestine, stroking the mucosa increases the strength of the rhythmic contractions on the oral side. In the rabbit's small intestine, stroking is ineffective but longitudinal stretching causes strong contractions of the circular muscles, also exclusively on the oral side. The contractions arise above the stimulated region but may be conducted in either direction. Local application of nicotine sulfate in concentrations as low as 1:20,000 blocks the spread of the response orally. It is concluded that an enteric synaptic mechanism conducting impulses chiefly or exclusively orally is responsible for the polar responses of the intestine. In the dog, the introduction of a bolus induces, on the oral side, strong rhythmic activity of the same frequency as that of the empty organ. Action potentials are similar to those associated with the activity of the empty intestine. The evidence of this and previous work indicates that the individual contractions are myogenic but that the strength of the contractions can be modified by an enteric reflex mechanism.

**The effect of morphine on urinary volume in women** J. T. BRADBURY, O. F. KRAUSHAAR (by invitation), W. E. BROWN (by invitation) *Dept of Obstetrics and Gynecology, State University of Iowa, Iowa City, Iowa*. This is a clinical report of the effect of morphine on the urinary output in ten (10) women. The patients studied represented various adult ages, and multiple observations were obtained on each patient. Each patient had nothing by mouth from midnight until 3 p.m. on experimental days. A retention catheter was inserted at 7 a.m. and the intravenous administration of fluids begun. In each case 2,400 cc of fluid was given intravenously, and the rate was controlled so that

the total volume was introduced into the vein in approximately 5 hours. The experiments were done on days when the patient was afebrile and clinically seemed to be in water balance. Hourly urines were collected for 8 hours and the volume and specific gravity determined. The urine for the next 16 hours was collected as a single specimen. On the test days, Morphine Sulfate gr  $\frac{1}{4}$  I.M. was given to each patient at the time the intravenous fluids were begun, regardless of age or weight.

The hourly urine volumes were markedly diminished after morphine was given so that the average 8 hour excretion volume was one half that on control days, 890 cc as compared to 1770 cc. The urine flow increased 8 or 9 hours after the morphine was given so that the total excretion in 24 hours was similar on control and morphine days.

**The secretory pressure of the liver of the chicken** N. R. BRUNER (introduced by A. B. LUCKHARDT) and R. L. GUNNING *Dept of Physiology, The University of Chicago*. This report covers sixteen recordings of the bile pressure from the hepatic duct. The maximum pressures obtained were 253, 152, 165, 140, 125, 190, 119, 175, 147, 186, 160, 172, 180, 190, 173 and 167 mm of water. This gives an average maximum pressure of 168 mm of water and a range of 119 to 253 mm of water. Rhythmic fluctuations of pressure were noted in all cases. These fluctuations were from 10 to 30 mm of water and occurred every one to five minutes. Upon observation of the hepatic duct it was found that this pressure variation was due to peristaltic action along the duct. After the bile pressure reached the above peaks, a gradual decline in pressure was observed. The first nine experiments were made on chickens anesthetized with sodium barbital. The last seven chickens were anesthetized by positive pressure ether insufflation. In the first nine experiments the hepatic duct was cannulated about a centimeter from its entrance to the intestine. In the last seven experiments, besides cannulating the hepatic duct as in the first experiments, the small connecting duct between the liver and the gall bladder was clamped off with a hemostat close to the liver.

**Thermodynamic analysis of the relative effectiveness of narcotics** F. BRINK and J. M. POSTERNAK (by invitation) *Johnson Foundation, University of Pennsylvania*. In a multiphase system in equilibrium with respect to a particular constituent its thermodynamic activity in experimentally inaccessible phases equals that measured in another phase. This principle is useful in analysis of chemical effects on living cells where only concentrations in extracellular phases are known. Since narcosis is a process apparently reaching equilibrium this method is applicable to systematic study of the relative effectiveness of narcotics. Ferguson (*Proc Roy Soc B*, 127: 387,

1939) reported that in contrast to greater than thousandfold variation in aqueous concentrations of equally effective doses of many substances the corresponding thermodynamic activities lie in a relatively narrow range of values. The present calculations show that for many narcotics of different chemical structure, acting on several cell types, equal degrees of effect are produced at approximately equal thermodynamic activities. This conclusion contrasts sharply with findings of Ferguson and of Bridger (*Nature*, 158: 585, 1946) both of whom emphasize the progressive increase, in an homologous series of compounds, of the thermodynamic activity at which narcosis occurs. It seems that both of these systematic relations among the activities of narcotic substances can occur. (In these calculations the pure narcotic at the same temperature is chosen as the standard state.)

New experimental evidence to be presented confirms the existence, for some cell types, of the law that equal degrees of narcosis are produced at equal thermodynamic activities.

Circulatory reserves shown by animals under acceleratory exposure. S. W. BRITTON, and V. A. PERTZOFF (by invitation), *University of Virginia Physiological Laboratory*. Reduction in carotid arterial pressure observed during exposure to acceleratory forces may be considered as a blood pressure deficiency area in time. Such a deficiency area is directly proportional to the product of the intensity and duration of centrifugation. Used in correlation with post acceleratory blood pressure changes, a significant evaluation of an animal's circulatory reserve condition may be obtained.

Monkeys and dogs were tested at  $\frac{1}{2}g$  for 5-30 sec. Of the two factors involved, animals appeared to be more sensitive to intensity than to time of exposure to  $g$  forces. Comparison of the effects of fasting, exercise, cold, anesthetics, hemorrhage, operative and other conditions was made with the responses of the normal animal. Marked deficiencies in recoverability were observed by the circulatory index method, even after mild acceleratory exposures only were employed.

Food intake as a mechanism of temperature regulation in rats. JOHN R. BROBECK, *Laby of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* That food intake varies with environmental temperature is a widely accepted principle, and it is generally assumed that this relationship is derived from the fact that total energy expenditure varies with temperature. Thus, food intake is thought to be determined by total energy expenditure. The following experiments suggest, however, another interpretation. Adult male rats were acclimatized to 84°F, and then exposed for 18 hours to any one of several temperatures ranging from 65° to 97°. Food intake, water intake, weight change and body temperature

change were measured. At the lower temperatures (65°-72°F), food intake increased, and body temperature rose by about 1.0°F (presumably because of the animals' greater alertness and activity at the end of the exposure period). A similar rise in body temperature was observed at the other end of the temperature scale, i.e., at 94°F, but here the food intake was extremely low, and the animals lost weight because their expenditure was so much greater than their energy intake.

At these two levels (70° and 94°F) body temperature change was the same and total energy expenditures were comparable, yet food intake was entirely different. These data suggest that under the conditions of these experiments food intake served as a mechanism of temperature regulation.

The production of waves of inhibition in the esophageal-fundic region. DANIEL A. BRODY (by invitation), J. D. LAWSON (by invitation) and J. P. QUIGLEY, *Department of Physiology, University of Tennessee, Memphis, Tennessee*. In Bayliss and Starling's original demonstration of the Law of the Intestine the presence of a relatively large foreign body in the intestine was essential to the production of the wave of inhibition preceding the wave of contraction. Similarly, we have observed that deglutition regularly produced a transient fall of intraluminal pressure within both the fundus and lower esophagus in 8 out of 10 human subjects (74 of 78 trials) when the pressure recording device was in these two regions. However, when recording tips were only in the stomach, deglutition produced no significant pressure change in the body and fundus. In the former situation, the esophageal pressure (measured by the open tube-optical manometer technic of Brody and Quigley, *Gastroenterology*, In Press) began to fall 0-3 seconds after the onset of swallowing, fell 5-15 cm. of water within 4 seconds and rose to normal in approximately 10 seconds. This negative pressure wave was immediately followed by an elevation of pressure in the esophagus to 10-35 cm. of water above the basal level and then returned to normal in 6-9 seconds. The negative pressure wave 22-45 mm. distally in the fundus began 0-2 seconds after the onset of the esophageal negative wave, fell 3-12 cm. of water, persisted 10-14 seconds, then returned to normal. Succeeding positive waves did not exceed 5 cm. of water in amplitude. Perhaps the wave of inhibition preceding the wave of contraction is a reserve mechanism elicited when the contraction wave alone is inadequate to propel a bolus.

Measurement of gastric tonus in the normal human. DANIEL A. BRODY (by invitation) and J. P. QUIGLEY, *Department of Physiology, University of Tennessee, Memphis, Tennessee*. Gastric tonus (the tension exerted by a given number of muscle fibers, Brody and Quigley, *Bull. Math.*



Biophysics, In Press) was determined in five normal human subjects. The method consisted of (a) recording the intraluminal pressure (Brody and Qugley, Gastroenterology, In Press) of the 6 hour fasting stomach distended to a standard volume of 600 cc, (b) measuring the pressure decrease following the administration of three drops of nitroglycerine sublingually or amyl nitrite by inhalation until severe head throbbing developed, (c) solving the equation  $T = Pr/2$ , where  $T$  = wall tension/cm (tone),  $P$  = fall in pressure and  $r$  = 5.24 cm, the radius of a 600 cc sphere. The distended stomach is not entirely spherical and its wall is not uniform, thus the calculated values of tone are approximate. However, this method appears to supply the most accurate measure of tone under physiological conditions so far developed. The fall in basal intragastric pressure occasioned by nitrites was 3.0-7.3 cm of water, corresponding to a reduction of tone of 7.3-19.1 gm. Assuming the complete abolition of tone by nitrites, then the original gastric tone was 7.3-19.1 gm. In general, the magnitude and duration of tone reduction was inversely proportional to the degree of gastric phasic activity which preceded the nitrite administration. In one completely vagotomized subject, exposed to 18 hours constant Wangensteen drainage, nitrites lowered gastric tone 14.8 gm.

**Insulin inactivation by tissue extracts** R. H. BROTH-KAHN (by invitation) and I. ARTHUR MIRSKEY *May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio*. A system capable of inactivating insulin during *in vitro* incubation has been demonstrated in rat, rabbit, beef and human livers. In the rat, this system has also been found in smaller amounts in the kidney and in muscle, plasma appears to contain none. The muscle of the rabbit is a poorer source than rat muscle. The insulin inactivator is extracted after homogenization of the freshly excised tissues in 3 volumes of ice water and is found in the supernatant fluid from the centrifuged homogenate. The active fraction can be precipitated by adjustment to pH 5.0 and partially recovered by redissolving at pH 7.5. The inactivator from rat liver is believed to have the properties of an enzyme. It is destroyed by heating to 70° C for 10 minutes. Its maximum activity during incubation is demonstrated at pH 6 to pH 8. The effect of the temperature during incubation has not been thoroughly investigated although activity is observed at 37° C and not during incubation at 5° C. At 37° C and pH 7.5 the destruction of insulin proceeds at a fairly constant rate for the first part of the incubation and then decreases progressively. Dialysis of rat liver extracts results in a loss of activity. The activity of such a preparation can be recovered by the addition of magnesium or manganese salts. The activity of an undialyzed extract can be almost completely

inhibited by low concentrations of cupric and zinc salts but is not affected by salts of magnesium, manganese, calcium or sodium  $10^{-3}$  M iodocetate and iodosobenzoate also inhibit its activity. This inactivator is not believed to have the properties of known proteolytic enzymes since the extracts do not produce appreciable splitting of a hemoglobin substrate at pH 7.5 nor is their activity inhibited by the relatively non specific protease inhibitors of the soybean.

**The sequence of functional changes in a neurone during narcosis and anoxia** D. W. BROOK, F. BRINK and M. G. LARRABEE *Johnson Foundation, University of Pennsylvania*. It is the purpose of this communication to consider how a number of related characteristics of nerve are modified by narcosis, anoxia and certain ions.

Thus, if chlorotone be applied to frog axons, a series of changes ensue with respect to time and concentration. Under low concentrations there is first the well known rise in threshold. Under higher concentrations a still greater stability of the nerve is revealed by its inability to initiate rhythmically recurring impulses under the stimulus of a chemical agent such as sodium citrate. This region which has lost the power to initiate impulses can however conduct impulses which originate in another portion of the nerve. A still higher degree of stability, induced by higher concentrations of chlorotone, are necessary to abolish conduction. Analogous, perhaps, is the observation that synaptic excitation in a ganglion is blocked under lower concentrations than is axonal conduction.

Further evidence of these progressive alterations in the properties of nerve are found in the changes in oxygen utilization in the time course of the extra oxygen consumption of activity and in the resting oxygen consumption.

The inter-relation of these several functional characteristics of nerve which are thus progressively modified will be considered in terms of the relative actions of anoxia and various ions as well as of narcotics.

**Antidromic potential recordings from the medullary pyramid of the cat** JOHN M. BROOKHART and RUSSELL E. MORRIS (by invitation) *Institute of Neurology, Northwestern University Medical School, Chicago, Ill.* As a foundation for projected studies of the pyramidal system, recordings have been derived from the medullary pyramids of cats anesthetized with Dial or anemically decerebrated. Antidromic activity has been initiated in these fibers by stimulating electrodes placed in the region of the lateral cortico spinal tracts at various cord levels. Sharply localized pickup from the medullary pyramids is possible using a monopolar needle electrode oriented in a Horsley-Clarke instrument.

Due largely to the impossibility of exciting all

cortico spinal fibers simultaneously, the resultant recordings are not strictly uniform since the active fibers vary in distance from the recording electrode. Features common to all recordings include (1) a prominent deflection initiated at an indicated conduction velocity of approximately 35 m p s, reaching a peak at an indicated velocity of 14-20 m p s, (2) one or more subsequent elevations reaching their peaks at indicated velocities between 10 and 5 m p s, (3) a total duration of discharge varying between 10 and 20 ms. Sporadically, small elevations are seen having indicated velocities of 85-100 m p s and 40-50 m p s. Indications of the slowest fiber responses are obtained only with conduction distances of less than 50 mm. It has thus far been impossible to activate the pyramid antidromically with cord stimuli applied caudal to the upper thoracic levels. Fiber diameter measurements indicate that the great mass of fibers lies in the 1.0-4.0  $\mu$  range with only scattered fibers exceeding 5  $\mu$ , the largest measured being 11  $\mu$  in diameter.

**Responses of inhibited motoneurons**  
CHANDLER MCC BROOKS, J C ECCLES (by invitation) and J L MALCOLM (by invitation) *From the Departments of Physiology, The University of Otago, Dunedin, N Z, and the Johns Hopkins University, Baltimore, Md.* Inhibition of reflex discharge of cat's gastrocnemius or quadriceps motoneurons has been effected either directly by a centripetal volley in the large proprioceptor fibres of the antagonistic muscles or more indirectly by a volley in other afferent nerves of that limb (proprioceptive or cutaneous). The testing responses of such motoneurons have been evoked either by monosynaptic excitation (the myotatic reflex) or by volleys fired antidromically along their motor axons.

(i) Inhibition has no appreciable action on the initial rate of rise of the pure synaptic potential (recorded either from ventral root or by focal electrode), but it lowers the summit and expedites the decay' (ii) Furthermore inhibition increases the critical voltage at which a synaptic potential generates a reflex discharge (iii) Motoneurons may be deeply inhibited and yet show no depression of the propagation of antidromic impulses into their somas, i.e. of the soma spike potential (confirming Renshaw) (iv) Nevertheless inhibition depresses the facilitation of the soma spike potential that occurs during a synaptic potential (Brooks and Eccles, *J Neurophysiol*, 1947, 10: 251), this depression paralleling effect (i) above.

These observations accord well with the hypothesis that inhibition is produced by focal areas of anelectrotonus on the motoneuron's soma (Brooks and Eccles, *Nature* 1947, 159: 760). Such areas would limit the spread and fusion of the

local responses set up under the excitatory synaptic knobs, and hence produce effects (i) and (ii). On the contrary, antidromic propagation into the soma will be mediated by currents flowing through widely spreading circuits and so should be little if at all depressed (effect iii) by the postulated anelectrotonic foci, particularly when the complementary dispersed catelectrotonic areas are taken into account. Effect (iv) derives from (i).

**Enzyme inhibitors on electric activity of frog brain**  
V B BROOKS, R E RANSMEIER, (introduced by R W GERARD) *Dept of Physiology, The University of Chicago*. The electrical activity of the isolated frog brain, both the spontaneous normal rhythm and convulsive type caffeine spikes (Libet, B, and Gerard, R W, *J Neurophysiol*, 1941, 4: 438), was utilized to study some relationships between metabolism and brain activity.

The anti-cholinesterase, tetraethylpyrophosphate (TEP)  $10^{-7}$  or  $10^{-3}$  M) markedly increases the amplitude and regularity of the normal 4-6 a sec rhythm, and maintains this for a long period (Compare ACH eserine, Gerard, R W, *Ohio J Sci* 41: 160, 1941). Di-isopropylfluorophosphate (DFP) ( $10^{-4}$  M), but not TEP, may slow the waves to two a sec and render them more spike like. DFP ( $10^{-4}$  M), but not TEP ( $10^{-3}$  M), completely suppresses spikes otherwise obtained with caffeine alone. The metabolic inhibitor, methylfluoroacetate (0.05M), depresses the amplitude of the spontaneous rhythm, but leaving the frequency unchanged, and reduces the period of surviving activity of the excised brain to about one tenth of normal. Methylfluoroacetate, like DFP, fully suppresses caffeine spikes. Sodium fluoroacetate (0.05M) has no significant effect on the spontaneous or caffeine-induced activity, presumably because of poor penetration (see abstract Boyarsky, et al). Experiments with respiratory intermediary compounds will be discussed.

**The pulmonary arterial pressure of the chicken**  
F BROWN (by invitation) and S ROBBARD *From the Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Illinois*. We have recently investigated the nature of the body temperature systemic blood pressure relationship which is seen in frogs, turtles, mammals and birds. In these animals a lowering of the body temperature results in a fall in arterial pressure, increasing the temperature results in a return of the pressure to normal.

The pulmonary arterial pressure of the turtle, being essentially the same as the systemic arterial pressure, also varies with the changes in body temperature. It was therefore decided to determine if similar changes occurred in the pulmonary arterial pressure of a warm blooded animal as body temperature was varied. The normal pulmonary arterial pressure of the chicken was found to

20/8 mm Hg. This is approximately the pulmonary pressure reported for mammals. The warming or cooling of the chicken had no effect on the pulmonary pressure although the systemic arterial pressure did change significantly. Neither acetylcholine nor epinephrine had any significant effect on pulmonary arterial pressure, as in the mammals. It would thus appear that the temperature pressure relationship does not apply to the pulmonary arterial circulation in the bird. Furthermore, these results show that the pulmonary arterial pressure remains at about the same level in warm blooded as in cold blooded vertebrates while the systemic arterial pressure rises considerably above that in cold blooded vertebrates.

**Effect of tetra-ethyl-ammonium chloride on intra-arterial blood pressure in patients with coarctation of the aorta.** GEORGE E. BROWN, JR. (by invitation) and EARL H. WOOD, *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota.* Intraradial and intrafemoral arterial pressures of twelve patients with coarctation of the aorta were recorded continuously by hypodermic strain gage manometers. Simultaneous records of ear pulse, blood content of the ear, heart rate, respiration, and saphenous venous pressure as well as electrocardiograms, were obtained, in most instances. Continuous records were obtained with the persons in the supine position and during tilting to the 65 degree upright position for periods of fifteen to one hundred twenty seconds. Tetra-ethyl ammonium chloride was injected intravenously in doses of 4.4 to 7.7 mg. per kilogram of body weight. Continuous records were obtained with the patients supine and during intermittent tilting for periods up to sixty minutes after injection of the drug. After injection of the drug with the patients supine, maximal decreases of intraradial pressure below average values obtained in the control period averaged 36 (12-69) mm. of mercury, systolic, and 8 (-1-19) mm. of mercury, diastolic. Intrafemoral pressure decreased an average maximum of 22 (6-38) mm. of mercury, systolic, and 6 (-3-20) mm. of mercury, diastolic. Postural hypotension was observed in these patients after administration of the drug. In seven normal persons studied under similar circumstances, the average maximal decrease in radial systolic pressure produced by the drug was 24 (14-36) mm. of mercury. The average decrease in diastolic pressure was 1 (-12-14) mm. of mercury. Figures in parentheses are extreme values.

**A method of assaying the efficacy of resuscitating procedures.** MARSHALL BRUCER (by invitation) and H. G. SWANN, *Dept. of Physiology, University of Texas Medical School, Galveston.* Any method of assay of a resuscitating procedure demands an accurate prediction of the proximity of death. Among all methods studied—blood gas analyses

and respiratory and cardiac behavior—only a characteristic collapse of systolic pressure presaged imminent death with accuracy. Employing optical recording of arterial blood pressure which could be continuously inspected, various resuscitating procedures were applied at different points during the collapse of systolic pressure.

In anoxia from breathing 1% CO in air, rhythmic positive pressure insufflation of the lungs with O<sub>2</sub> was superior to both air insufflation and manual artificial respiration. In anoxia from breathing N<sub>2</sub>, insufflation with 5% CO<sub>2</sub> in O<sub>2</sub>, or the Emerson "Resuscitator" using O<sub>2</sub>, were superior to manual artificial respiration. In anoxia from breathing 2-13% O<sub>2</sub> in N<sub>2</sub>, insufflation with O<sub>2</sub> or with 5% CO in O<sub>2</sub>, or with the Emerson "Resuscitator" were all superior to manual artificial respiration.

In each of the three types of anoxia studied, resuscitation was successful if begun early but failed if begun late in the period of systolic collapse. There was an intermediate zone during which heart and respiration recovered but only with residual dementia in the animal. The time span between early and late collapse is a matter of seconds—so short that unless resuscitation machinery is immediately available, in practical emergencies the manual method is probably as efficacious as any so far studied.

**The uropepsin output in cats given histamine-caffeine in beeswax.** GRADY R. BUCHER and ARTHUR ANDERSON, *Department of Biochemistry, Vanderbilt University, Nashville.* The purpose of this investigation was to learn if uropepsin output in cats is related to gastric hypersecretion stimulated by the daily administration of histamine-caffeine in beeswax. Two series of experiments were performed. These differed in diet and histamine dose. In the first, the diet contained a 9% protein, the daily drug dose was 150 mg. caffeine (alkaloid) with 0.5 mg. histamine base (diphosphate). In the second there was about 19% protein and 150 mg. caffeine with 0.75 mg. histamine base (dihydrochloride). In the first study, no difference in the average 24-hour uropepsin output of 19 control days and 14 test days was found. One cat that died in convulsions had developed a gastric ulcer. Three months later the cats were used in the second study. After 10 days on the low protein diet and 9 days on the high protein diet, the injections were resumed for 22 days. The uropepsin output was significantly increased in all cats following the change to the high protein diet. In the first eight days of the dose period, all cats increased their outputs still further. In two the increase was statistically significant. Only one of the cats maintained the significantly higher output, the other sickened, refused its food, and the uropepsin output fell off. It appears that uropepsin output is related more closely to the protein consumption

of the animal than to the stimulation of gastric secretion by the use of histamine caffeine mixture in beeswax.

**Consciousness and unconsciousness during anesthesia in relation to brain potential** W E BURGE *Department of Physiology, University of Illinois, Urbana* When one platinum electrode was placed on the forehead and another on the forearm with a galvanometer connected in the circuit the beam of light was deflected on the galvanometer scale in such direction as to indicate that the forehead was positive to the forearm in 1680 subjects, principally University students and faculty members. Similarly, the forehead was found to be positive to the forearm in 174 hospital patients prior to anesthetization and operation. During anesthetization scalp potential was decreased to zero. In deep anesthesia (plane 3) polarity was reversed in most of the patients, that is, the scalp became negative to the forearm. Upon recovery from anesthesia polarity was again reversed and the forehead became positive to the forearm.

It has been shown (Anesthesiology, Vol 6, No 1, 1945) in dogs that the positive potential of the scalp fluctuated with the negative potential of the underlying brain cortex so scalp potential may be used as an index to brain potential. Hence, the positive potential of the scalp observed in the conscious human indicated that the underlying brain cortex was negative, the decrease during anesthesia indicated diminution in brain potential, and the reversal in polarity in deep anesthesia indicated that the brain cortex had become positive. These observations suggest that consciousness and unconsciousness may depend on electrical condition of the brain cortex, consciousness being associated with a gain of electrons and negative condition, an unconsciousness with a loss of electrons and a positive, or less negative, brain cortex.

**Epithelial movements in woundhealing in frog corneas** WILHELM BUSCHKE *Ayer Foundation Ophthalmic Research Laboratory, Manhattan Eye, Ear, and Throat Hospital, New York* Epithelial movements described previously by many authors as a primary mechanism of epithelial wound-repair, have been studied in detail on stained whole thickness flat preparations of the cornea in frogs. Pinprick injuries heal in vivo and in vitro with the typical radial orientation of the marginal epithelia seen previously in rats (Friedenwald and Buschke, *Jl Cell & Compur Physiol* 23, 95, 1944; Buschke, *Fed Proc* 6, 85, 1947). The latent period between injury and onset of orientation is inversely related to temperature with a temperature coefficient of  $Q_{10} = 3$ , i.e. lower than in the rat ( $Q_{10} = 5$ ), but still of a magnitude as to suggest the importance of metabolic processes. The temperature optimum of the process is between 5 and 25°, i.e. about 15° below that in the rat. Two

ment and shaping of the individual cells of the woundmargin are seen. One type consists in the extension of pseudopods into the woundzone which eventually join those of other epithelia. This type of movement is seen in the basal layers around pinpricks and in later stages of the healing of larger injuries. The second type of movement consists in a flattening of the marginal cells tangentially to the woundmargin leading to a microscopically continuous often arcade like outline of the epithelial sheet, this type of movement is seen in the upper epithelial layers around pinpricks and in the early stages in larger injuries and scratches. Pseudopodial processes are most marked in cells which for some portion of their surfaces are separated by an optically clear space from neighboring cells. This suggests that some correlation exists between the spatial relations of neighboring cells of the epithelial sheet on the one hand, and the mode of movement and shaping the individual cell follows in the healing process at different stages and with different sizes and shapes of the injury on the other hand.

**Comparative lipotropic activity of parenterally administered pancreatic extracts in dietary fatty livers** J F CANEPA (by invitation) and A C IRY *Dept of Clinical Science, Univ of Illinois, College of Medicine, Chicago* The lipotropic activity of both Dragstedt's and Chaikoff's pancreatic extracts was tested by the parenteral route on adult white rats kept on a high fat (40%) low-protein (5%) diet. This diet is known to produce fatty infiltration of the liver in these animals when fed for 20 days. The pancreatic extracts were prepared according to the techniques described by the aforementioned authors. Three groups of 10 rats each (5 males and 5 females) were used. The extracts, dissolved in saline, were injected subcutaneously every day in the following doses: Group I—167 mgm of Dragstedt's lipocaine per rat, Group II—30 mgm of Chaikoff's fraction C27 per rat, Group III—control. After 20 days the animals were sacrificed and the livers removed and analyzed for fatty acids, phospholipids and cholesterol. The average total fatty acids content of the liver of the three groups of animals was found to be 3.88, 15.4, and 15.8%, respectively. Similar though not so striking differences were also noted in the phospholipids and cholesterol values. These results show that Dragstedt's and Chaikoff's extracts, though both active by oral administration in the depancreatized dog, behave differently when injected subcutaneously in the rat. Therefore, we may conclude that the lipotropic principle present in these pancreatic extracts is not the same.

**Effect of diet on the in vitro synthesis of alamine** by liver ATTILIO CANZANELLI, DAVID RALPH and RUTH GUILD (by invitation) *Dept Physiology, Tufts College Medical School*

ing liver slices from control rats maintained on a standard synthetic diet formed  $7.11 \pm 0.26 \mu\text{g}$  of  $\text{NH}_4\text{N}/\text{mg}$  dry tissue/hr when incubated at  $37^\circ\text{C}$  for two hours in a Krebs-Henseleit medium containing  $0.1 \text{ M}$  pyruvate and  $0.04 \text{ M}$  ammonium carbonate. Livers from rats initially on the standard diet and then fasted for 12, 24, 48, 72 and 96 hours, gave the following values respectively  $6.43 \pm 0.91$ ,  $1.17 \pm 0.15$ ,  $1.10 \pm 0.14$ ,  $2.59 \pm 0.65$  and  $2.69 \pm 1.04$ . When rats were placed on a diet containing no protein, but high in carbohydrate for 24 hours the amount synthesized was  $8.08 \pm 1.02$ , and for 72 hours was  $8.48 \pm 0.57$ . On a non protein, high fat diet for 24 hours, the livers gave  $6.19 \pm 0.35$ . With a diet containing no fat but high in carbohydrate, values of  $8.20 \pm 0.33$ ,  $7.09 \pm 0.87$  and  $6.79 \pm 1.13$  were obtained for feeding periods of 24 hours, 48 hours and 72 hours respectively. On a diet without carbohydrate but high in fat the figures were  $3.28 \pm 0.69$  and  $2.82 \pm 0.44$  for 24 and 72 hour periods. A value of  $3.77 \pm 0.41$  was obtained on a 24 hour diet containing no carbohydrate but high in protein. It appears that fasting markedly reduces the capacity of liver slices to synthesize alanine and that this reduction is due to carbohydrate deficiency.

**A method for direct measurement of rate of oxygen utilization by nerve** F. D. CARLSON (by invitation), F. BRINK, and D. W. BROOK Johnson Foundation, University of Pennsylvania. For studying the  $\text{QO}_2$  of peripheral nerve we have developed a respirometer in which fluid of known oxygen concentration is drawn at a constant rate through a capillary tube containing a length of nerve and past an oxygen electrode. Variations in oxygen consumption of the nerve cause variations in the oxygen remaining in the solution which passes the electrode. The  $\text{QO}_2$  of the nerve is given by

$$\text{QO}_2 = \frac{(i_0 - i_n)F}{k \cdot W} \frac{\text{moles}}{\text{gm hr}}$$

where  $k$  is oxygen concentration sensitivity of the electrode,  $i_0$  and  $i_n$  electrode currents for empty and nerve filled chamber respectively,  $W$  nerve weight and  $F$  flow rate. Using respirometers having  $k$  values of  $10^3$  microamperes per molar solution of  $\text{O}_2$  and a flow rate of  $2.2 \times 10^{-4}$  liter/hr the change in  $\text{QO}_2$  ( $2 \times 10^{-7}$  moles/gm hr) accompanying 10/sec stimulation of 9 milligrams of frog sciatic nerve is readily detected. By means of a "bucking circuit" the useful  $\text{QO}_2$  sensitivity can be increased tenfold, the limit being set by stability of flow rate and electrode sensitivity.

Electrode linearity and sensitivity must be established for each experimental solution. Slow drifts in sensitivity can be minimized by periodically interrupting electrode current.

Advantages of this respirometer are: continuous

recording, scale reading proportional to  $\text{QO}_2$ , facility of changing solutions, ease of making functional measurements on nerve, maintenance of constant chemical environment.

**An apparatus for the measurement of pulmonary function** L. D. CARLSON, A. W. MARTIN, and V. GATTONI (by invitation) Department of Physiology and Biophysics, School of Medicine, University of Washington, Seattle 5, Washington. An apparatus has been assembled for determining alterations of normal pulmonary ventilation in terms of rate of flow, volume of flow, pulmonary emptying rate, residual and reserve air, and oxygen consumption. A flow-meter using a 100 mesh screen as an orifice and recording through a Stratham strain gage is interposed between the patient and a Sanborn metabolism apparatus. Gas samples for a Lilly nitrogen meter are drawn from the mouthpiece. Records are made on the kymograph drum and a recording oscillograph. A special attachment at the mouthpiece allows the entire metabolism apparatus to be filled with 100% oxygen. By taking simultaneous flow, nitrogen concentration, and metabolism records, the degree of pulmonary mixing can be seen in the first three breaths, and residual plus reserve air calculated after two minutes. Subsequent measurement of reserve air and other lung volume components complete the data for description of a large number of the phenomena involved in respiration. The apparatus may be used in assessing the value of treatment of asthma. The apparatus may be adapted to bronchospirography (Jacobdeus, *et al*). The degree of correlation with other methods has not been completely worked out, but the accuracy is adequate for general clinical and experimental tests.

**A method for studying reflex activity under varying conditions** L. D. CARLSON and A. W. MARTIN (with design in electronics by R. S. Bark) Department of Physiology and Biophysics, School of Medicine, University of Washington, Seattle 5, Washington. This report describes (1) a simple apparatus suitable for classroom use for classroom use for eliciting a stretch reflex, and recording electrically the isometric responses of the muscle, by means of strain gauges, together with the summated action potentials in the muscle or nerve supplying it, (2) preliminary experiments on the reflex response of a soleus muscle of the cat to stretch and ipsilateral peroneal nerve stimulation under varying conditions. A standardized procedure was used to evaluate the effects of a variety of conditions, *e.g.*, drug action, on reflex arcs of increasing complexity. Muscle tension was recorded from the soleus muscle of decerebrate and spinal cats and muscle action potentials were recorded with coaxial or bipolar electrodes. A stretch reflex was elicited in decerebrate and spinal cats

with a 3 mm stretch during one second. In decerebrate animals, pentothal in remarkably small doses (0.0026 gm/kg) abolished the reflex. The convulsant barbiturate (0.0004 gm/kg) first augmented then depressed the reflex. In spinal animals (24 hours transected) the myotatic hyporeflexia made analysis difficult. Again, the convulsant barbiturate first augmented and then depressed the stretch reflex and induced spontaneous rhythmical activity. Stimulation of the peroneal nerve (50/sec) inhibited the stretch reflex and induced a post stimulatory rebound in the decerebrate, but not in the spinal animal. The rebound (ipsilateral extension) is augmented by pentothal (0.0013 gm/kg), and then depressed (0.00625 gm/kg). The convulsant barbiturate augmented the effect at 0.0004 gm/kg.

**Cell fractionation and gonadotrophin assays of anterior pituitary glands.** HUBERT R. CATCHPOLE, *Dept. of Pathology, Univ. of Illinois, College of Medicine, Chicago.* Anterior pituitary glands of sheep, collected on ice, were subjected to cell fractionation by various methods. Following grinding in a mortar with 5 volumes of saline at pH 9.0 (Clude), cell debris and nuclei were removed by centrifugation at 1500 g (repeated 3 times). Large granules were separated from the extract by centrifugation at 18000 g for 5 minutes (repeated 3 times) and a microsomal fraction obtained by centrifugation of the remaining supernatant fluid at 18000 g for 90 minutes. All operations were conducted at 0°C. Satisfactory preparations of nuclei were obtained from the original precipitates by washing and sedimentation. All fractions were also obtained by breaking the cells in a Waring Blender at a slightly acid pH (6.0) as described in the method of Dounce. Gonadotrophin assays were made on fractions, washings and supernates, using immature rats and mice. Little or no activity appeared in the final supernate by the 'alkaline' method, whereas the 'acid' method extracted about  $\frac{1}{3}$  of the total measured activity. In both preparations, gonadotrophin activity was heavily concentrated in the large granule fractions. Based on a given equivalent of fresh tissue, microsomes contained only a small fraction ( $<1/10$  -  $<1/50$ ) of the activity of large granules, possibly representing contamination with active particles of an intermediate size. Nuclei showed a low activity, compatible with contamination by adherent large particles.

**The distribution of response thresholds in studies of insect chemoreception.** L. E. CHADWICK and V. G. DETHIER (by invitation) *Medical Division, Army Chemical Corps, Army Chemical Center Maryland, and the Johns Hopkins University.* Data are presented to show that the distribution in an insect population of acceptance and rejection thresholds for chemicals is normal with respect

to the logarithm of concentration. The significance of this observation is discussed in relation to the planning of experiments and the interpretation of results.

**Changes in blood level of citric acid and calcium in nephrectomized dogs.** T. S. CHANG (by invitation) and SMITH FREEMAN, *Department of Experimental Medicine, Northwestern University, Chicago, Ill. and Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.* Blood levels of citric acid and calcium were studied before and after bilateral nephrectomy in dogs. Before the operation the average value of citric acid in the plasma was 5.25 mg per cent while that for serum calcium was 11.5 mg per cent. Blood was analyzed every 24 hours after the operation. It was found that both constituents showed an increase, an average of the 24-hour values for citric acid and calcium were 20.3 mg per cent and 13.52 mg per cent, respectively. The average values on 5 dogs 48 hours after nephrectomy were 24.5 mg per cent for citric acid and 12.76 mg per cent for calcium. Forty eight hours after nephrectomy, 40 mg of citric acid per kilo body weight was injected intravenously drop-wise in one hour. Blood samples were taken immediately after injection as well as one, three, and twenty-four hours later. The average values for citric acid and calcium are shown in the following table.

|             | End of<br>injection<br>mg % | 1 hour<br>after<br>mg % | 3 hours<br>after<br>mg % | 24 hours<br>after<br>mg % |
|-------------|-----------------------------|-------------------------|--------------------------|---------------------------|
| Citric acid | 45.4                        | 42.9                    | 50.1                     | 39.9                      |
| Calcium     | 14.10                       | 14.0                    | 15.0                     | 12.1                      |

Citric acid remained high but calcium decreased to 10.45 mg per cent before the death of the animals. The dogs survived from 70 to 165 hours after nephrectomy, the average survival period was 114 hours.

**The effect of CO upon the ventilation response of the beaver.** JOHN L. CHAPIN (by invitation) and HERMANN RAHN, *Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York.* In his review of the physiology of the diving mammals Irving has shown that in general these animals have a very slight ventilatory response to CO compared to non-diving mammals. The inhalation of 10% CO<sub>2</sub> by the anesthetized beaver is reported as having a very slight effect. In order to rule out the possible depressing effects of the anesthesia experiments were conducted on a 12 kg unanesthetized beaver. A special mask was built and the ventilation, expired CO<sub>2</sub> and O<sub>2</sub>, and breathing rate continuously recorded. The minute ventilation during the control period was compared with the 6th-10th minute after breathing the various CO<sub>2</sub> mixtures started

The ventilation increase was as follows with 3.8% CO<sub>2</sub> in air—27%, with 6.5% CO<sub>2</sub> in air—81%, and with 11.4% CO<sub>2</sub> in air—252%. These values are somewhat lower than those obtained on unanesthetized dogs in this laboratory, and differ considerably from those obtained on man.

**Effect of exercise on renal plasma flow** CARLTON B. CHAPMAN (by invitation), AUSTIN IRNSCHHEL, JOHN MINCKLER and ANCEL KRIS *Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis*. In order to obtain quantitative information on the effect of work on renal plasma flow in the normal subject we have devised a method which permits measurements to be made before, during, and after sustained exercise. The clearance substance employed is para amino hippurate and the analytic method that of Bratton and Marshall. The exercise consists of 2 consecutive 15 minute periods on the treadmill. Three levels of exercise have been investigated: A 3 mph on the level, B 3 mph at 5 per cent grade, and C 3.5 mph at 10 per cent grade. All 3 levels produce a progressive decline in renal plasma flow, and the extent of the decrease is a function of duration and severity of the exercise (table 1).

| Level | % decline, 15 min | % decline, 30 min | No subjects | No tests |
|-------|-------------------|-------------------|-------------|----------|
| A     | 7.5               | 17.0              | 3           | 6        |
| B     | 17.6              | 26.6              | 9           | 14       |
| C     | 20.1              | 33.2              | 2           | 4        |

Return of renal plasma flow to basal values after cessation of exercise is much slower than pulse and respiratory rates and blood pressure. After the mildest of 3 levels of exercise, return to basal values was incomplete in most subjects after 40 minutes of rest, although the residual deficit at that time was not great. It was progressively greater after the same interval of rest following the other 2, more severe, exercise loads. With the heaviest work load, recovery was still incomplete one hour after termination of exercise.

**The role of the vagi in the crossed phrenic phenomenon** P. O. CHATFIELD and S. MEAD (introduced by E. M. Landis) *Dept. of Physiology, Harvard Medical School, Boston, Mass.* The term "crossed phrenic phenomenon" refers to the recovery of activity in a hemidiaphragm paralyzed by hemisection of the spinal cord above C3, when the contralateral functioning phrenic nerve is cut or blocked in various ways. Previous investigators have shown that the crossing of descending respiratory impulses can occur on section of the vagi alone. This finding was confirmed during the present experiments performed on rabbits. Crossing was produced also by activation of Hering-Breuer

afferents by partial occlusion of a tracheal cannula and by high frequency stimulation of the central end of the cut vagus. Low frequency vagal stimulation inhibited crossing. After bilateral vagal section had produced crossing, an increase in crossing still occurred after inactivation of the functioning phrenic. No afferents inhibitory to crossing could be demonstrated in the cut phrenic. It was concluded that in certain instances crossing may be produced by changes in afferent vagal impulses brought about by inactivation of the functioning phrenic.

**Derivation of leads I and III in the dog from analysis of unipolar limb leads** H. M. CHERNOFF (by invitation), W. KAUFMAN (by invitation) and L. H. NATHAN *Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.* It has been shown that in any unipolar limb lead certain recognized ("proximal") regions of the heart when depolarized produce downward deflections in the electrocardiogram, while other ("distal") regions when depolarized produce upward deflections. Lead I is derived by subtracting the potentials of Vr from those of V1. It follows that activation of regions of the heart resulting in similar and equal deflections in each of these unipolar leads will not be recorded in Lead I. Such regions are the anterior right and posterior left ventricle, a small portion of upper left anterior ventricle near the septum. Conversely, regions whose activation results in opposite deflections in Vr and V1 will be maximally recorded in Lead I. Such regions are the right posterior and greater part of the left anterior ventricles. Lead III is derived by subtracting the potentials of V1 from those of V6. Activation of regions resulting in similar and equal deflections in each of these unipolar leads will not be recorded in Lead III. Such regions are right posterior and greater part of left anterior ventricles. Activation of regions resulting in opposite deflections in V1 and V6 will record maximally in Lead III. Such regions are the right anterior ventricle, right and left apex, left posterior ventricle, and small portion of upper left anterior ventricle near septum. This study confirms and amplifies the results of previous experiments on the nature of Leads I and III in the dog.

**Ephedrine effect on human uterine contractions** GEORGE P. CHILDS (by invitation), B. S. HARDMAN (by invitation), R. A. WOODBURY and R. TORPIN (by invitation) *Departments of Pharmacology and Obstetrics and Gynecology, University of Georgia, School of Medicine, Augusta, Georgia*. The effect of ephedrine sulphate on uterine contractions was studied in normal women and in women with dysmenorrhea at various periods during the menstrual cycle. From 25 to 50 mgm. were given i.v. and the contractions recorded by changes in intra-uterine balloon pressures. There were

three balloons in the uterus and one in the cervix, each containing one cc of water. Ephedrine either reduced uterine activity or produced a change in the rhythm of contractions converting disorganized into organized contractions. In the latter case the initial response was the production of one to five slow contractions in the upper segment of the uterus followed by a lower segment contraction wave. The uniform pattern then ensued and was maintained for some time. Many of the patients who were in pain prior to the ephedrine were relieved. The relief of the pain may possibly be attributed to the effect of ephedrine on the central nervous system, yet the uterine effect of the drug must play an important role since pitocin, which has no known CNS effects, influenced the uterus in a similar respect to that of ephedrine and also relieved the pain of dysmenorrhea, see Woodburn, Child, and Torpin these abstracts.

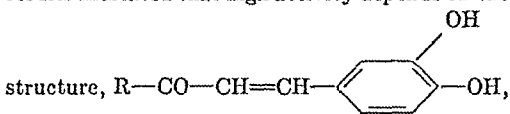
These results, therefore, suggest that the pain of dysmenorrhea may be relieved by drugs which cause or permit the uterus to contract with a uniform rhythmic pattern.

**Studies of the mechanism of increased chloride excretion during osmotic diuresis.** L. J. CIZEK (by invitation) and J. H. HOLMES, *Department of Physiology, College of Physicians and Surgeons, Columbia University*. The secretion of chloride in dogs was found to be increased during the diuresis produced by the continuous intravenous injection of either 50 per cent sucrose, 50 per cent glucose, 50 per cent sorbitol, or 10 per cent urea (12.5 cc per kilo at 2 cc per minute). The total amount of chloride excreted varied as the urine volume and bore no relation to the type of diuretic used. Creatinine clearances revealed that as the urine volume increased there was a decreasing reabsorption of chloride from the glomerular filtrate, reabsorption reaching its lowest point at the height of diuresis, returning toward normal as the urine volume decreased. The effect was unrelated to changes in filtration rate and was not modified by the administration of either pituitrin or "doca" (desoxycorticosterone). The same reduction in chloride reabsorption at the height of diuresis was obtained in dogs in which plasma chloride had previously been lowered by osmotic diuretics plus a salt free diet, and in dogs in which the plasma chloride level was raised during the experiments by infusing a solution containing 50% sucrose and 10% NaCl. It would appear that neither the type of diuretic, the serum chloride concentration, "doca" nor pituitrin modified the tubular activity.

**The lack of effect of estrogen on the sex skin of the infant male chimpanzee.** GEORGE CLARK, *Dept of Anatomy, Chicago Medical School, Chicago, Illinois* and *Yerkes Laboratories of Primate Biology, Orange Park, Florida*. In an earlier study, it was reported that the effects of estrogenic ther-

apy in the male chimpanzee differed from those reported by Gilbert in the chacma baboon. However, the baboons used were young adolescents, while the chimpanzees were young adults, so it seemed possible to explain this difference on the basis of age alone. Although adolescent chimpanzees were not available, it was possible to use two chimpanzees just over two years in age. One had been castrated at the age of ten weeks, while the other was intact. Both animals were given ethinyl estradiol (4.5 mg per day by mouth for 10 days). This dosage is 180 times that sufficient to produce full tumescence in the female castrate chimpanzee or if measured on a weight basis 900 times the threshold dose. In contrast to reported results for the male chacma baboon but confirming those for the adult chimpanzee no changes in sex skin were observed. There was no swelling of the perianal region, no edema and no reddening of the penis. It may be concluded that a true species difference exists.

**Molecular structure and activity of "vitamin P"-like substances.** Inhibition of oxidation of epinephrine. WILLIAM G. CLARK and T. A. GEISSMAN (by invitation). *Scrpps Metabolic Clinic, La Jolla, Calif* and *Dept of Chemistry, University of California, Los Angeles*. According to the theory of Lavollay, Parrot, et al (Helv Chim Acta, 29 1283, 1946), "vitamin P" acts *in vivo* to prolong the effects of epinephrine. Methods of assay based on this were developed in order to relate molecular configuration to physiological activity. Many flavanones, flavonols, flavones, chalcones and other miscellaneous orthodihydroxyphenolic compounds and metal-complexing agents and their derivatives were examined. The results indicated that high activity depends on the



where R can be, among other things, another hydroxyphenol if the OH groups are placed properly. The ortho hydroxy groupings must be free. Many compounds were found with markedly greater activity than rutin and "Citrin" (Szent Gyoergy). Methylated derivatives such as hesperidin were nearly devoid of activity. Activity probably is related to the reducing powers of the compounds, quinone formation and metal complexing or chelating capacity. In the light of these and other results it is doubtful that epinephrine antioxidant properties and capillary fragility ("permeability," "Filtration") are exclusively related so far as "vitamin P" effects are concerned.

**Effect of tetraethylammonium on responses of isolated intestine to angiotonin and other substances.** DEAN A. COLLINS, *Dept of Physiology, Temple Univ School of Medicine, Philadelphia*



Page and Taylor (Science, 105 622, 1947) have reported that the tetraethylammonium (TEA) ion increases responses of blood pressure to angiotonin, adrenalin, and usually to histamine, mecholyl, and barium chloride. In the present study the action of TEA on isolated guinea-pig ileum, suspended in Tyrode's solution at 38°C, has been investigated. Angiotonin preparations were made from heparinized dog's plasma incubated with renin and then heated at pH 5. The resulting fluid was treated with tribasic calcium phosphate which adsorbs some impurities. Further purification was effected by adsorption of the angiotonin on charcoal, elution with glacial acetic acid, and precipitation of the angiotonin with ethyl ether. The responses of the ileum to these angiotonin preparations were augmented by the presence of TEA bromide in dilutions from 1 in 13,000 to 1 in 40,000. Responses to histamine were also consistently increased by TEA. Responses to acetylcholine and barium chloride were only occasionally augmented, and were often depressed. That the augmentation of responses to the angiotonin solutions was not due to increased sensitivity to impurities is indicated by the relatively huge doses of control fluids required to elicit responses from the ileum. The control fluids were prepared from plasma by the same procedure used in the preparation of angiotonin except that incubation with renin was omitted.

A method for constant, long-term intravenous infusion of the unanesthetized dog. W. D. COLLINGS, C. J. MARTIN (by invitation) and G. R. WALTERS (by invitation). *Department of Physiology, State University of Iowa, Iowa City*. Apparatus has been

designed so that fluids may be infused continuously into a dog's jugular vein for as long as 28 days. The principal parts consist of two brass swivels basically constructed like stopcocks. The larger swivel is mounted in the top of the dog's cage, and the smaller one is attached to the back of the dog's neck with plaster roll. Infusion fluid, pumped by a Brewer automatic pipetting machine, enters the stationary portion of the large swivel. The rotating center portion is connected to the dog by plastic (Tygon) tubing which is attached to the rotating portion of the small swivel. The outlet of the stationary portion of the small swivel leads to 2 mm (O.D.) plastic (Tygon) tubing which passes subcutaneously to the jugular vein. This tubing is inserted into the lumen of the vein 4 to 8 inches. Thus, with two swivels freely moveable and with a system of heavy rubber bands to keep slackened tubing from wrapping about the dog's neck, there has been little tendency for the animal to become entangled. The equipment can be arranged so that the animal may be removed from the cage for other procedures, such as blood pressure measurement, without any interruption of fluid administration.

Measurements of rapid changes in oxygen consumption by nerve following brief periods of stimulation. C. M. CONNELLY (by invitation) and D. W. BRONK. *Johnson Foundation, University of Pennsylvania*. It has been possible to record an increase in the oxygen consumption of *Limulus* leg nerves that begins within less than 5 seconds from the start of stimulation. Furthermore, it has been possible to detect an increase in oxygen consumption resulting from the conduction of but a single volley.

The speed and sensitivity necessary for such measurements have been obtained by placing an oxygen electrode (Davies and Brink, *R. S. I.* 13, 524, 1942) in direct contact with the nerve. The geometry is such that the oxygen consumption of the resting nerve is balanced by the inward diffusion of oxygen, and the electrode current, which is a measure of the oxygen tension, is steady.

The increased oxygen consumption resulting from the conduction of one or more volleys of impulses causes a decrease in oxygen tension at the electrode. For a brief period of time the change in oxygen tension is a direct measure of the oxygen consumption of the nerve. Thereafter, the oxygen tension is modified by inward diffusion of the gas and the rate of oxygen consumption must be determined by calculation. Accordingly, the method is of especial value for determining the time course of the beginning of the extra oxygen consumption of activity.

Embolization of platelet agglutination thrombi in the hamster's pouch produced by heparin. ALFRED LEWIS COPLEY. *Marine Biological Lab., Woods Hole, Mass.* and *Lab. of Cellular Physiology, Dept. of Biology, New York Univ., New York*. Earlier investigations demonstrated that heparin produces thrombocytopenia in vivo and a decrease of single platelets in vitro. These findings were explained by Copley and Houlihan (*Blood*, Suppl. 1, 182, 1947) who showed that isolated platelets were agglutinated when incubated with heparin plasma. We found that systemic or local heparin injection produced white emboli in the capillary bed of the nictitating membrane of the rabbit. In this investigation we observed the same phenomenon of white thromboembolization in the cheek pouch of the hamster following systemic injections of sodium heparin. This phenomenon was a constant finding in the 32 hamsters studied, and was not observed in the control animals systemically injected with physiologic saline. Immediately following the appearance of these emboli, blood samples were secured directly from the heart, and placed between cover slips. Numerous small white emboli of about leukocyte size were observed in undiluted blood and blood diluted with isotonic sodium citrate solution 1:10. These emboli which consisted of platelet agglutinates were of the same size

as those in the capillary circulation. Sections of the cheek pouch also revealed, upon Giemsa staining, platelet agglutinates as well as single platelets. Heparin, although highly active as an anticoagulant, does not prevent platelet agglutination, but induces it, which accounts for the thrombocytopenia.

**Pilot metabolism and respiratory activity during varied flight tasks.** E. L. COREN, *Physiological Laboratory, University of Virginia Medical School*. Determinations of metabolism (103) and respiratory activity (332) were made during varied flight tasks in both a Link trainer and a Piper J-3 airplane. Ten subjects were used, so selected as to represent a variety of flight experience. Caloric output was found to increase with increasing complexity of flight task (straight and level, turns, patterns, rough air) in all subjects, and to be greater in inexperienced as compared with highly experienced pilots. Since these differences were evident even when a specific task demanded no appreciable increase in manipulation of the controls (turns, climbing turns), it was concluded that the raised energy outputs observed represented an increased generalized muscular tension with increased task complexity, further, that this tension was related to pilot experience. The subjects exhibited an increased respiratory rate with an increase in task complexity, although there was considerable individual variation. This respiratory sensitivity to alterations in flight pattern was not, however, invariably correlated with experience. As in the case of caloric output, changes in respiratory rate were not directly correlated with control movements, and the altered respiratory patterns observed were attributed to changing demands in mental activity, resulting in altered, generalized muscular tension.

The results obtained in the J-3 airplane complemented those secured with the Link trainer, and it was concluded that the latter type of study is qualitatively valid for aircraft, with the additional feature of permitting much more rigid control. It was further concluded that determination of pilot caloric output is a valid and convenient indication of the work demand imposed by various flight tasks.

**The influence of sodium salts on the extracellular space in experimental hypoproteinemc edema.** SAMUEL A. CORSON and ELIZABETH O'LEARY, with the technical assistance of Opal Cain. *Department of Physiology, School of Medicine, University of Minnesota and Howard University*. Experiments were performed on 3 trained unanesthetized dogs rendered hypoproteinemc by a combination of a low-protein diet and massive plasmaphereses. The plasma proteins fell from an average normal value of 6.6 g % to an average of 3.4 g %. The extracellular space was determined by the thiocta-

nate method (in the postabsorptive state). Repeated administration of NaCl either orally (1 g/kg. body weight) or intravenously (10 ml/kg of a 1 S, 2 O, or 2 S M solution delivered by a constant infusion pump at the rate of 2.5 ml/min) produced no change or led to a decrease in the extracellular space. In only 4 out of more than 20 experiments was there any increase in the extracellular space following NaCl administration. This increase happened to coincide with a significant fall in the hematocrit. Whether there is any causal relationship between the anemia and the change in extracellular space remains to be determined.

**Neural control of the renal shunt.** J. H. COYT (by invitation) and DONALD H. BARRON, *Laboratory of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Observations on the neural control of the renal shunt indicate that 1) the efferent path is via the splanchnic roots (T10-12), 2) the initial response in rabbits and cats to stimulation of a spinal afferent is unilateral, 3) with continued stimulation the response becomes bilateral in 5-10 minutes in cats, and 3-4 5 hours in rabbits, 4) the crossing occurs in the cord, 5) the anuria which follows an approximation of the crush syndrome in cats can be relieved by novocainization of either splanchnics or the spinal roots T10-12.

**Observations on the oxytocic property of human blood.** ARTHUR A. COX (introduced by R. R. Overman), *Department of Physiology, University of Tennessee, College of Medicine, Memphis, Tennessee*. It has been reported that blood from patients in labor exerts an oxytocic effect upon the isolated guinea pig uterus, (Compt. Rend. Soc. de Biol. 107: 88, 1931). We have studied the oxytocic effect of human blood upon the isolated rat uterus suspended in Tyrode's solution in a 37°C constant temperature bath. Blood from pregnant patients, from males, and from non-pregnant females has been studied. When blood from any of these three groups of patients is added to the uterine bath within five minutes after withdrawal, each cc of blood is found to exert an oxytocic effect equal to approximately 0.01 units of pitocin. After incubation at 37°C for one hour, blood from males and non-pregnant females showed no diminution of oxytocic activity, whereas blood from pregnant patients showed a marked reduction of this activity. These results demonstrate the presence of a substance in pregnant blood which is capable of destroying this oxytocic principle. It has been reported previously by others (Am. J. Obst. and Gynec. 52: 1014, 1946) that pitocin when added to pregnant blood is destroyed by an enzyme, which has been called pitocinase. Blood from males and non-pregnant females does not possess this enzyme. Since pitocinase appears in the blood only during pregnancy and since only in pregnant blood were we able to demonstrate a diminution of oxytocic

activity on incubation, it seems reasonable to conclude that at least a portion of this oxytoxic activity is due to the presence of the posterior pituitary hormone

**Analysis of combined effects of exercise and carbon dioxide inhalation on pulmonary ventilation in man** F N CRAIG, JANE STUBBS (by invitation) and F N MARZULLI (by invitation) *Physiology Section, Medical Division, Army Chemical Center, Maryland* Normal male subjects inhaled air and mixtures of 2.6 per cent carbon dioxide in air and of 5.0 per cent carbon dioxide in air, from a 600 liter spirometer for periods of three minutes while sitting in a chair, walking at 4.0 miles per hour and running at 5.8 miles per hour on a level treadmill in a room at 20°C and 40 per cent relative humidity

The increments of ventilation rate in liters per minute due to either exercise or carbon dioxide inhalation beyond the rate under reference conditions, were augmented by the introduction of the other factor. The ratio of ventilation rate augmented by either factor to the ventilation rate at reference conditions, was diminished by the introduction of the other factor.

The ventilation rate observed when both factors were present together was compared with the rate calculated from the effect of each factor operating alone (1) by adding the increments over the reference condition due to each factor and (2) by multiplying the ratio of exercise augmented rate to reference rate by the ratio of carbon dioxide augmented rate to reference rate.

Calculated rate as per cent of observed  $\pm$  extreme deviation

| Reference Condition          | Augmented Condition         | Incremental Method | Method of Ratios |
|------------------------------|-----------------------------|--------------------|------------------|
| sitting, air                 | running, 5% CO <sub>2</sub> | 81 $\pm$ 5         | 141 $\pm$ 23     |
| walking 2.6% CO <sub>2</sub> | running, 5% CO              | 92 $\pm$ 8         | 100 $\pm$ 11     |
| walking air                  | running, 5% CO <sub>2</sub> | 91 $\pm$ 7         | 105 $\pm$ 9      |
| sitting 2.6% CO <sub>2</sub> | running, 5% CO <sub>2</sub> | 90 $\pm$ 4         | 129 $\pm$ 20     |

#### The effect of anesthetics on calcium release

MARY CREGAR (introduced by L V Heilbrunn) *Zoological Laboratory, University of Pennsylvania* Experiments were performed in an attempt to investigate the calcium release theory of anesthesia and stimulation. Muscle fibers were removed from frog gastrocnemii and weighed. Half the fibers were immersed for one hour in a solution of 2% ether in frog Ringer and half (controls) were immersed in a frog Ringer solution for the same time. The fibers were then removed and a sample of the immersion fluid was analyzed for calcium. In 9 out of the 10 experiments performed, more calcium was found in the immersion fluid containing ether than in the control fluid. Similar experiments were performed using solutions of 2% cocaine in Ringer and 0.4%

chloroform in Ringer. In 7 of the 13 experiments with cocaine, more calcium was found in the cocaine-Ringer solution than in the control, in 4 no difference could be found between the cocaine-Ringer solution and the control, while in 2, more calcium was found in the Ringer solution. Of the 10 experiments performed using chloroform, 7 showed more calcium present in the chloroform-Ringer solution, while in 3 the same amount of calcium was found in both the chloroform and the control solutions. In 6 of the experiments using chloroform the muscle fibers were analyzed for calcium. In 5 of the 6, more calcium was found in the fibers immersed in Ringer than in those immersed in chloroform-Ringer, the sixth showed no difference. In general these results seem to support the calcium release theory.

**Errors induced by phosphate in flame photometer analysis of tissue ash for sodium and potassium** J M CRISMON, *Department of Physiology, Stanford Univ School of Med* In the course of parallel analyses of muscle tissue ash for sodium by the method of Butler and Tutthill (*J Biol Chem* 61:523, 1921) and with the flame photometer, agreement was obtained by the two methods when the dissolved tissue ash was subjected to treatment with CaO for the purpose of phosphate precipitation. Analysis of solutions of tissue ash not subjected to phosphate precipitation yielded values for sodium and potassium appreciably lower than those obtained gravimetrically or with the flame photometer after phosphate precipitation. Additional studies made on muscle ash from rabbits, cats, and rats showed that the presence of phosphate lowered the apparent Na concentration by 24 to 53 per cent and the apparent K concentration by 19 to 29 per cent. Solutions designed to contain 50.0 m Eq per liter of Na were made from Reagent Grade or C P Samples of NaCl, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, Na<sub>2</sub>HPO<sub>4</sub>, Na<sub>2</sub>PO<sub>4</sub>·12H<sub>2</sub>O, and Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub>·10H<sub>2</sub>O. To each solution was added 10 m Eq per liter of K in the form of KCl. Aliquots of each solution were diluted 10 fold, a portion of each was treated with CaO to precipitate phosphates, and the portions, with and without phosphate, were analyzed in the flame photometer for sodium and potassium. For all of the phosphate salts the apparent sodium concentration was approximately 50 per cent of the value measured after the precipitation of phosphate. Potassium concentrations were not altered by the presence of phosphate in the above mixtures. Flame photometer analysis of urine should probably also involve elimination of phosphate.

**The effect of inanition on pituitary-adrenal function in the guinea pig** SAVINO A D'ANGELO, ALBERT S GORDON, and HARRY A CHARAPPLA, *Department of Biology, Washington Square College of Arts and Sciences, New York University* A pre-

vious study had indicated that immature guinea pigs fed a normal diet to a body weight of 300-350 grams and then completely starved or chronically underfed display adrenal enlargement characterized by hypertrophy of the inner cortical zones and atrophy of the glomerulosa, thyroid regression, and marked structural changes in the basophiles of the anterior hypophysis (Anat Rec 81 suppl no 184, 185, 1941). The present experiments were designed to test, in the starved animal, the generally accepted thesis that cortical hypertrophy is mediated largely through the cortico trophic mechanism of the anterior pituitary, and that suppression of this mechanism results in cortical atrophy. Female guinea pigs at a body weight of 300-325 grams were placed on a complete starvation regime (water ad lib) and given daily injections, for 5-6 days, of either 2-5 cc of a saline cortical extract or 2.5-15.0 milligrams of desoxycorticosterone. It was found that the administration of these cortical substances to the starved guinea pig failed to prevent cortical hypertrophy in animals losing 30-50% of their body weight, and failed to alter appreciably the course of the body weight loss. In contrast, guinea pigs hypophysectomized for 2-9 days with corresponding degrees of weight loss showed cortical atrophy. Further attempts are being made to inhibit the adrenal enlargement of starvation by the administration of much higher doses of potent cortical materials. The significance of the cytological changes in the anterior hypophysis as related to the cortical hypertrophy in starvation will be discussed.

**Alterations in the rate of glycolysis in human blood following the addition of salts** T. S. DANOWSKI, *Department of Research Medicine and the Renziehausen Foundation, University of Pittsburgh School of Medicine*. The disappearance of glucose during the incubation of defibrinated human blood at 36 to 37°C was measured in a series of experiments. The addition of NaCl in amounts sufficient to increase its concentration in blood by 40 meq per liter had no effect on glycolysis. Additions of CaCl<sub>2</sub> which increased the concentration by 40 or by 20 meq per liter invariably retarded glycolysis, while increments of 2.5 meq per liter were without effect. Equivalent amounts of KCl or MgCl<sub>2</sub> only rarely slowed the disappearance of glucose. Glycolysis was accelerated, on the other hand, by the addition of NH<sub>4</sub>Cl producing increments as small as 2.5 meq per liter. The introduction of HPO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>-</sup>, and of HCO<sub>3</sub><sup>-</sup> as the sodium salt hastened in each instance the disappearance of glucose. The accelerating effects on glycolysis of the HPO<sub>4</sub><sup>-</sup> and the NH<sub>4</sub><sup>+</sup> ions appear to be additive, since glucose disappeared more rapidly from blood which contained added (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> than it did from blood with equivalent amounts of either NH<sub>4</sub>Cl or Na<sub>2</sub>HPO<sub>4</sub>.

**The time course of in vivo oxygen consumption of cerebral cortex following electrical stimulation** P. W. DAVIES (by invitation), R. G. GRENNELL and D. W. BRONK *Johnson Foundation, Univ of Pennsylvania*. The oxygen cathode has been employed to make in vivo measurements of local oxygen consumption at the surface of the cerebral cortex after a period of electrical stimulation. The experiments were performed under moderate Dial anaesthesia on cats with a trephine hole over the supra sylvian gyrus. Oxygen consumptions were taken at intervals by measuring the rate of fall of oxygen tension when the oxygen cathode was suddenly pressed sufficiently hard to occlude the local circulation.

At the end of a strong stimulus (100 shocks/sec, ten seconds duration, 40 v open circuit at the output of the isolating transformer) the rate of oxygen consumption is usually twice that obtained before stimulation. Within a half minute, however, the rate falls to about two thirds of the "resting" value. By this time also there is a marked reddening of the venous blood. Afterwards there is a period of recovery during which the oxygen consumption rises gradually to the "resting" rate. This period may be as short as two minutes but is often longer. A much weaker stimulus (4 v) of the same frequency and duration causes hardly any increase in oxygen consumption, but leads to the same reduction and recovery experienced with the strong stimulation. The relation of these findings to those of Dusser de Barenne and McCulloch on facilitation and extinction, and to those of de León on spreading depression, will be considered.

**Thermal irritation of alimentary mucosa** ROBERT E. DAVIS (by invitation) and A. C. IVER, *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago*. A subject of particular interest in the genesis of peptic ulcer and carcinoma is the possibility that ingestion of hot substances may irritate the mucosa of the upper alimentary tract. The present study is an attempt to correlate degrees of heat injurious to mucosa with that to which the human subject exposes himself. Temperature tolerance was determined in 120 medical students by measuring the maximum temperature at which they would drink coffee in rapidly successive swallows. The average temperature was 57.8° Centigrade, with a range from 51°C to 68°C. The standard error for this series was 0.26°C. Subjects with temperature tolerance of 68°C ingested successively 50 cc, 100 cc, and 200 cc of water at that temperature, and intra gastric temperatures were measured by means of a gastric thermacouple. The maximum temperature, 49.9°C, was reached within ninety seconds with rapid cooling and return to normal intragastric temperature within 30 minutes. In acute experiments on dogs, gastric, esophageal, and buccal mucosae were bathed with

water at graded temperatures, and biopsies taken. Coagulation and superficial sloughing of gastric mucosa were demonstrated only at 65°C or above. Similar changes occurred in esophageal mucosa at 60°C or above. Buccal mucosa blistered and sloughed at the relatively low temperature of 55°C, which is within the range of human temperature tolerance. It remains to be proven whether or not the animal thresholds for thermal injury obtain in the human subject and whether repeated exposure of the stomach to a temperature of 50°C will cause histological changes.

**Influence of intravenous cytochrome c upon visual acuity of the dark adapted human eye**  
 STANLEY K. DAVIS (introduced by J. W. Heim) *Aero Medical Laboratory Wright Field, Dayton, Ohio*. Mitigation of effects induced by hypoxia, both anemic and anoxic, through parenteral administration of cytochrome c in animals and man has been reported in recent months by Proger and Dekaneas (*Science*, October 25, 1946, pp. 389-390). No corroborative studies have as yet reached publication, but two failures to duplicate their results in animals (rats) have been reported (Schemberg and Michel (*Science*, April 5, pp. 365-366), Stadie and Marsh (*J. Clin. Invest.* 26: 899)). No further reports on human use have yet appeared. The present study was designed to determine any effect of intravenously administered cytochrome c (Wyeth) upon the visual acuity of the dark adapted eye under hypoxic conditions. Seven human subjects were subjected in "blacked out" altitude chambers to atmospheric pressures equivalent to 18,000 feet or a total of 35 exposures of one-hour duration each. Each subject was pre adapted before each exposure until basal visual thresholds were achieved. Thresholds were determined every 10 minutes during exposures with a portable Hecht-Schlar Adaptometer (Cenco) and were recorded as logarithms of the light intensity just sufficient to permit identification of a figure interposed against the light source. Intravenous cytochrome c solutions (50-100 mg) and colored saline were given alternately prior to exposure of each subject. Subjects were kept ignorant of the content of injections. Oxygen tension in the chamber was checked continuously with a Pauling Oxygen Meter.

With the method described above, it was not possible to demonstrate significant differences in the rate or degree of deterioration in visual acuity in human subjects prepared with and without intravenous cytochrome c (Wyeth).

**Adrenaline hyperglycemia in hypophysectomized dogs**  
 R. C. DE BODO, I. H. SLATER (by invitation), H. F. WEISBERG (by invitation), K. F. PRILSCOTT (by invitation) *Department of Pharmacology, New York University College of Medicine*. As previously shown, hypophysectomized

dogs with adequate amounts of liver glycogen show a smaller hyperglycemic response to intravenous adrenaline than normal dogs. In the present work, the development of this altered response, its relation to insulin hypersensitivity and glucose utilization was studied. The test dose of adrenaline, 0.0035 mg/kg/min for five minutes intravenously, causes a marked hyperglycemia (maximum rise 13-81, average 58 mg per cent) within fifteen minutes from the start of the infusion. Four-five days after hypophysectomy the adrenaline hyperglycemia is distinctly decreased and becomes gradually less pronounced as the postoperative time increases. After four weeks the hyperglycemic response is slight. This phenomenon seems to develop parallel with the hypersensitivity to insulin as described by Slater et al in these Proceedings. When the hypophysectomized animal has reached the stage at which the adrenaline hyperglycemia is greatly reduced, the intravenous infusion of glucose produces a marked and prolonged hyperglycemia demonstrating that the abnormality cannot be attributed to increased utilization of the glucose liberated by adrenaline but must rather be related to the deficient mobilization of liver glycogen. Furthermore, when the blood sugar of normal animals is maintained at a low level by large doses of insulin, adrenaline still produces considerable hyperglycemia (maximum rise 31-46 mg per cent). This is further evidence that increased insulin activity is not the cause of the decreased adrenaline hyperglycemia noted in hypophysectomized animals. The relationship of this resistance to the hyperglycemic action of adrenaline to the various endocrine organs is being examined and will be discussed.

**The rod calorimeter**  
 RONALD DEERING (introduced by A. B. HERTZMAN) *Department of Physiology, St. Louis University Medical School*. The rate of transfer of heat from deeper tissues to a limited area (1 cm<sup>2</sup>) of skin surface may be estimated by means of a specially designed rod calorimeter. The principle of the instrument's design is based on the flow of heat in metal rods as expressed by Fourier's equation. Thermally insulated rods of aluminum, copper, or silver are employed as the heat conductor between the body surface and a reference temperature mass of either water or metal. The temperature difference of the rod ends is a function of the heat flowing in the rod and is measured by a thermocouple-galvanometer arrangement calibrated to read in calories per minute. The physical factors which govern the operation of the instrument have been quantitated and will be described. When applied to equilibrium conditions in the finger, the rod calorimeter yields estimates of blood flow which agree with simultaneous estimation by the photoelectric plethysmograph and other calorimetric techniques. The instrument offers the possi-

bility of fractionating heat losses from the surface of the body on a regional basis

**Influence of protein hydrolysates on the production of nephrosclerosis and hypertension by anterior-pituitary preparations** By R. DE GRANDPRÉ (by invitation), J. L. PRADO (by invitation), P. DONNICK (by invitation), J. LEDUC (by invitation) and H. SÉLIE. *From the Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montréal, Canada.* Following our previous studies concerning the influence of diets on the production of hypertension and nephrosclerosis by lyophilized anterior-pituitary (LAP) overdosage, we investigated the effect of various protein hydrolysates in the food. Experiments on black and white ♂ rats (sensitized by unilateral nephrectomy, castration and a high NaCl diet), treated with LAP, showed that diets whose nitrogenous constituents were 15% of casein plus an equivalent amount of protein hydrolysate were as effective in producing hypertension and nephrosclerosis as a ration containing 30% casein. "Essen amine" (a partially degraded lactalbumin), "Paranamine" (almost complete acid casein hydrolysate), "Amigen" (enzymatic casein hydrolysate) and "Bacto-peptone" added to a basic 15% casein diet were the ingredients used. In an additional group of 8 rats, the total amount of nitrogen in the diet was furnished as the hydrolysate "Amigen" and even here a high incidence of hypertension and nephrosclerosis was obtained. All the hydrolysates tried were approximately equally effective in inducing nephrosclerotic changes and hypertension.

It is tentatively concluded that the artificial hydrolysis of proteins does not destroy the factors indispensable for the production of anterior pituitary hypertension and nephrosclerosis, hence these could be the amino acids contained in the protein molecule. However in LAP treated rats receiving a diet which contained 15% casein and one of the following amino acids in an amount corresponding to that contained in a 30% casein diet, nephrosclerosis did not ensue: glutamic acid, glycine, methionine, cystine, tryptophane, valine, histidine, lysine, leucine, phenylalanine, aspartic acid, isoleucine, norleucine, arginine.

**Mechanism of Inactivation of  $\alpha$ -estradiol by rat liver "in vitro"** R. H. DE MEIO (by invitation), A. E. RAKOFF (by invitation), A. CANTAROW and K. E. PASCHAS. *Jefferson Medical College, Philadelphia, Pa.* The inactivation was studied incubating either male rat liver slices (100 mg.) or homogenate (10 per cent) in 5 ml. of Krebs solution, phosphate buffer pH 7.4, without glucose for two hours at 37.5°C. Usually 1  $\gamma$  of  $\alpha$ -estradiol per milliliter was used. The amount of biologically active material present at the end was determined by bioassay in mice. Complete inactivation by liver slices was observed whether the gas phase

was oxygen or air. In nitrogen or with boiled slices no inactivation took place. The 10 per cent homogenate prepared with the Waring blender or by crushing the frozen liver in a mortar to a fine powder produced from 40 to 70 per cent inactivation. No change in the behavior of the homogenate was observed when the Fuhrman and Crismon "intracellular medium" was substituted for the Krebs solution or when boiled liver extract or glucose was added to the medium. The inactivation by liver slices inhibited by caprylic alcohol, but not by cyanide, azide, thiourea, malonate, fluoride or monoiodoacetate. The addition of methylene blue to slices incubated in nitrogen brought about a 40 to 70 per cent inactivation. Nicotinamide and DPN added to the homogenate increased the inactivation by approximately 50 per cent. Our results indicate that no appreciable inactivation is due to conjugation of the estradiol. As a consequence of our findings we may conclude that the system cytochrome cytochrome oxidase does not participate, or if it does, only to a minor extent, and that the inactivation is probably produced by a dehydrogenating system or systems.

**The spread of muscle action potentials from active to inactive areas** J. S. DENSLAW and DAVID M. GRAHAM SERVICE (introduced by IRVIN M. KORR). *Still Memorial Research Trust, Kirksville, Missouri.* The presence, absence and degree of muscle action potentials shown in the electromyogram appear to depend on (a) the number of units (or fibers in denervated muscle) which are active and (b) the characteristics and placement of electrodes which are used. The present studies were done to determine the most effective method of measuring spatial differences between active and inactive muscle by the electromyogram. Needles enamelled to  $\pm 1$  mm. of the tip were inserted into the hind limb muscles of the decerebrate cat and paired with other enamelled or bare needles. Certain muscles were denervated and others were left intact. The following observations were made: (A) That with an enamelled electrode in an innervated muscle and one in a contiguous denervated muscle, the electrodes being less than 3 mm. apart and paired with a common reference electrode, the action potential from an active single unit or group of fibers in the innervated muscle might or might not pass the epimysium of both muscles and be recorded from the electrode in the denervated muscle. (B) When the action potential from an active unit in the innervated muscle was picked up in denervated muscle the voltage from the latter was usually extremely small. (C) When two electrodes were placed in an "electrically silent" denervated muscle and when there was massive contraction in nearby muscle, action potentials were observed which were indistinguishable in

character from those recorded from electrodes in the contracting muscles

**Liver regeneration in the rat** R W DENTON (by invitation) and A C Ivy *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago* This paper shows that liver tissue when fed to partially-hepatectomized rats facilitates the rate of regeneration of the rat's liver

The method of the experiment was to compare the dried weights of the liver mass gained in the control (fed dog food) and the "treated" (fed pigs' liver) groups Blues *et al* showed that the left lateral and median lobes constituted about 68.5% of the total liver mass We removed these lobes and calculated the 31.5% remaining The pigs' liver fed was coagulated by bringing it to a boil in order to reduce putrefaction and enhance its being eaten We present only data on rats not losing over 15 grams of body weight in either group No special pre- or post operative feeding was practiced, the rats ate *ad libitum*, and they were kept in separate cages After 10 days, the rats were sacrificed and the increment of liver obtained as the difference between the weight at necropsy and the calculated mass left in at operation In 29 control rats, averaging  $216 \pm 5.6$  grams body weight, the liver increment regenerated was  $1.144 \pm 0.03$  grams, and in 22 "treated" rats averaging  $218 \pm 7.2$  grams, the increment was  $1.652 \pm 0.06$  grams The difference of 0.508 grams was statistically "significant" by having a critical ratio of 7

The dry/wet weight ratios showed the regeneration livers to have returned at least to normal consistency No effect was obtained by injections of thiamine (200 micrograms/day) or with crude or purified antipernicious anemia liver extracts

**The stimulating effect of glycols and their polymers on the tarsal receptors of blowflies** V G DETHIER (by invitation) and L E CHADWICK *The Johns Hopkins University and the Medical Division, Army Chemical Corps, Army Chemical Center, Maryland* The stimulating effect of three homologous series of glycols on the tarsal receptors of blowflies has been studied by means of techniques previously used in the study of aliphatic acids and alcohols When the glycols are compared to the corresponding alcohols it is seen that the introduction of the second hydroxy group reduces the stimulating effectiveness of the compound Of the three series, that represented by the group ethylene, trimethylene, decamethylene is the most stimulating Members of the series represented by ethylene glycol through polyethylene glycol 1540 show increasing stimulative efficiency as the series is ascended

**A study of the effects on the intravenous injection of hypertonic solutions on the heart rates of cats and dogs** INCRITH J DEYRUP (by invitation) and WILLIAM W WALCOTT *Department of*

*Physiology, College of Physicians and Surgeons, Columbia University* Circulatory changes following intravenous injections of small volumes of 20% NaCl and 50% glucose have been studied in cats and dogs by means of femoral arterial pressure tracings One of the results of such injections is profound slowing of the heart, which is absent after bilateral vagotomy Various lines of evidence suggest that the effect depends on reflex, rather than direct stimulation of the medullary vagus center Thus, in cats the bradycardia was elicited consistently when 20% NaCl was injected into peripheral veins or into either the right or left auricular appendage, but was observed in only one out of six experiments in which hypertonic salt solution was injected into the ascending aorta Analysis of the latent period of the vagal response, particularly in comparison with the longer latent period of the gasp reflex following sodium cyanide injection, suggests that the bradycardia results from stimulation of receptors located, at least in part, within the heart itself Both the afferent and efferent neural path of the reflex are present in the vagi Thus, the effect is not abolished by removal of both sympathetic chains from the stellate ganglia to T<sub>7</sub>, but may be eliminated by injection of atropine sulfate

Reflex bradycardia following the injection of hypertonic solutions was observed in decerebrate cats and dogs, and in cats but not in dogs under nembutal It appears to be a reflex pattern related to the known cardiac adjustments resulting from stimulation of receptors in the venae cavae and in the heart itself

**The mechanics of Starling's law of the heart** JOSEPH R DIPALMA and RICHARD A REISS (by invitation) *Department of Physiology, Long Island College of Medicine, Brooklyn, N Y* Myographic measurements by two new techniques of the contractile force of the anaesthetized cat's heart were made Simultaneous recordings of the intraventricular pressure were also obtained With increased intraventricular pressure (clamping the aorta or pulmonary artery) the contractile force is increased Proof was obtained in the beating heart that cardiac muscle resembles skeletal muscle in regard to the phenomenon of initial tension This explains the fact that the contractile force of the heart muscle increases with a rise in intraventricular tension On the other hand, the contractile force decreases with increased venous return, and the opposite occurs with decreased venous return These latter findings are seemingly contradictory to the usual statement of Starling's Law of the Heart However, they do not contradict the original conception of the law as related to energy production The findings do indicate that the heart during systole follows the physical laws of an elastic sphere such as a soap bubble in which the contractile stress increases as the radius decreases Thus the force of

contraction of the heart muscle depends merely upon its initial length (diastolic size) not upon the initial stretch of muscle causing a greater subsequent contraction. Because of this fact the analogy which in the past has been drawn between the phenomenon of initial length of cardiac muscle as compared to that of skeletal muscle is not tenable.

**Observations on the peripheral circulation in neurogenic hypertension.** ISABELLE DOUGHERTY and SISTER M. A. C. DAY (introduced by A. B. HERTZMAN) *Department of Physiology, St. Louis University, School of Medicine.* Changes in the peripheral circulation of dogs during neurogenic hypertension have been studied. Photoelectric plethysmograms are recorded from various areas and pressure pulses are optically recorded from the femoral artery. The experiments are acute, the recording being continuous throughout the control period, the period of operation (removal of the buffer and sympathetic nerves, after Heymans) and the subsequent hypertensive period. The volume pulse amplitude can be correlated with the variations in pulse pressure. The blood pressure usually shows an immediate rise after the denervation, a rise to about 240/160 mm. Hg being obtained within 2 to 5 minutes. In some dogs the rise in blood pressure after the denervation is soon followed by a marked increase in volume pulses in the intestine and muscle, decreased blood pressure and death. In other dogs the hypertension develops more rapidly, rises to higher levels, and persists for hours. In such cases decreased volume pulses are usually seen in the skin, pad, intestine, and muscle. The anesthetic used influences the height of the rise in blood pressure.

**The basal metabolic rate of Georgia medical students.** PHILIP DOW and ROBERT H. SHULER (by invitation) *Dept. of Physiology, Univ. of Georgia School of Medicine, Augusta, Georgia.* Records have been kept of the basal metabolic rate determinations of the last ten sophomore medical classes (over 500 individuals). Procedures were carried out by the students on each other, techniques were supervised by the physiology teaching staff, and all records and calculations have been checked by the authors. A period of eight years is covered, including classes in all four seasons of the year.

The overall average runs between twelve and thirteen percent below the 1936 Mayo standards. This is lower than most reports from the South, but about the same as that recorded by Eaton in 1939 for a smaller group of similar age in New Orleans. Figures for age, sex, and seasonal differences are of questionable significance. Further statistics are in process of compilation. Limitations and implications of the findings will be discussed.

The authors wish particularly to emphasize the relevance of these results to the necessity for the use of local controls in forming diagnostic conclu-

sions. This caution has appeared repeatedly in the literature on metabolism, but unfortunately not in the instructions and tables furnished with clinical apparatus.

**Effect of convulsant agents on partially isolated neurones of the central nervous system.** C. G. DRAKE (by invitation), and G. W. STANAKI *Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada.* It is generally believed that the highest levels of the central nervous system are most sensitive to convulsants such as thujone, camphor and metrazol, and that decortication reduces the susceptibility of animals to convulsions. This concept is contrary to the "Law of Denervation" according to which isolated neurones should become more sensitive to chemical stimulating agents. With this in view the relative sensitivity of different regions of the central nervous system to various convulsant drugs was reinvestigated systematically in 37 sacrifice experiments on cats after aseptic semisection of the spinal cord or after semidecerebration carried out 4 days to 3 months previously, and in 28 chronic cats with removal of one motor cortex, a frontal lobe or a cerebral hemisphere. It was found that in both sets of experiments decentralized neurones reacted to smaller quantities of convulsant agents than intact ones, and that camphor, metrazol and picrotoxin evoked greater and more prolonged responses from decentralized neurones when sufficient time was allowed for sensitization to take place. This was ascertained on tracings of muscular contractions in sacrifice experiments and in moving camera recordings of convulsions induced in chronic animals. Chemically induced convulsions play a prominent part in the study of epilepsy and in the treatment of some mental derangements and it is felt that this investigation may contribute to the understanding of the mechanism of action of convulsant agents on the nervous system in which pathological processes are present.

**Acute effects upon the lungs of dogs of large intravenous doses of alpha-naphthyl thiourea (ANTU).** CECIL K. DRINKER and ESTHER HARDENBERGH (by invitation) *Dept. of Physiology, Harvard School of Public Health.* Dogs under nembutal anesthesia and given intravenously 2 per cent alpha-naphthyl thiourea (ANTU) in propylene glycol, 1 cc. per kilogram, develop fatal pulmonary edema. The first evidence of changes leading to edema is increase in the flow of lung lymph. Greater rate and minute volume of breathing follow. Blood gas analyses indicate that the hyperpnoea of rapidly developing pulmonary edema is peripheral in origin. Detailed protocols are given for two typical experiments.

**The effects of burns on kidney function.** ARTHUR J. DZIEMIAN (introduced by W. FLEISCHMAN) *Physiology Section, Medical Division, Army Chem-*



*ical Center, Maryland* Goats with severe third degree flame burns, covering about fifty percent of the body surface area, were studied. Kidney function, including glomerular filtration rate, effective renal plasma flow, filtration fraction and maximal tubular reabsorptive capacity, was determined in the normal animal, the burned animal, and burned goats treated with plasma or whole blood. Most severely affected was the effective renal plasma flow, which decreased in all burned animals. There were two classes of effects in regard to glomerular filtration rate and maximal tubular reabsorption after burning. In one group they decreased continuously until death. In the other group there was an initial sharp decrease in the filtration rate and reabsorptive capacity, followed by a return to normal or near normal values. Preterminally these latter values fell precipitously. Neither blood nor plasma infusions had any apparent effect on kidney function in the burned goat. The greater decrease in plasma flow as compared with filtration rate, with an increased filtration fraction, indicated a constriction of the efferent arterioles of the kidney.

**The influence of hypotension on coronary blood flow, cardiac oxygen metabolism and cardiac work** J E ECKENHOFF, J H HAFENSCHEIL, I L FOLTZ and R L DRIVLER (introduced by CARL I SCHMIDT) *Department of Pharmacology and Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania* The effect produced by hypotension upon coronary blood flow and cardiac oxygen metabolism has been measured in 8 intact dogs, lightly anesthetized with pentobarbital sodium. Coronary blood flow was estimated by the  $N_2O$  method, venous blood being collected by a catheter in the coronary sinus (Am J Med Sci, December 1947). Cardiac output was calculated by the Fick principle. Hypotension was produced by the intraspinal injection of procaine hydrochloride in 6 instances and by the intravenous injection of Etamon in 2 cases.

Blood pressure and cardiac output declined in all experiments. Coronary blood flow was essentially unchanged in three experiments in spite of considerable reductions in blood pressure and cardiac output. In three other dogs, coronary flow was reduced markedly, in these three instances, however, blood pressure and cardiac output were correspondingly markedly reduced. In the eight experiments blood pressure was decreased an average of 29% and cardiac output an average of 42%. These two factors were responsible for a 56% reduction in left ventricular work. Since coronary flow was diminished an average of 25%, it follows that coronary blood flow remained relatively excessive for the amount of work that the heart was performing in hypotension. The mechanical efficiency of the heart declined an average of 36%, but this does not mean that the heart was less able to perform the work demanded of it.

**A method for the study of gastric secretion in vitro** LESLIE J EDWARDS and CAROLYN TROWBRIDGE EDWARDS (introduced by R W RAMSEY) *Department of Physiology and Pharmacology, Medical College of Virginia, Richmond* An apparatus is described by means of which secretion of the isolated gastric mucosa can be studied in vitro. The method is, in principle, similar to that used by Gray (A J P 130 327, 1910) to study secretion by frog mucosa in that a piece of the mucosa, stripped away from the remainder of the stomach, is clamped between two separate chambers in such a way that the submucosal side is bathed by a nutrient medium containing any desired substrates, drugs, etc. In the present apparatus, the oxygen tension can be closely regulated to meet the requirements of the tissue and the true, unmodified secretion can be collected from the other chamber. The whole is mounted on a shaker in a water-bath at 37.5°C, making it suitable for study of secretion by warm-blooded animals. Under these conditions, the mucosa will live for at least three hours, as demonstrated by normal oxygen uptake from a Warburg flask at the completion of the experimental period. Secretion of both acid and pepsin has been obtained from the mucosa of rat and rabbit by this method. The apparatus is well adapted for study of the conditions necessary for gastric secretion and of the action of various stimulating and inhibiting agents directly upon the secreting cells.

**Factors influencing in vitro secretion of pepsin** CAROLYN TROWBRIDGE EDWARDS and LESLIE J EDWARDS (introduced by R W RAMSEY) *Department of Physiology and Pharmacology, Medical College of Virginia, Richmond* Gastric secretion from the isolated gastric mucosa was collected in vitro by the method described in the preceding communication. Studies were made using the rat and the rabbit, on the effect of variations of the composition of the nutrient medium (protein amino acids, etc.) on the production of pepsin. Further studies were made on the stimulating or inhibiting action of common autonomic drugs. Experiments indicate that nitrogen-containing compounds are necessary for the production of pepsin and that acetylcholine with eserine (and, to a lesser extent, eserine alone) stimulates its secretion in vitro.

**Further observations on intravascular temperature in man** LEON EISENBERG (by invitation) and H C BAZETT *Physiology Department, University of Pennsylvania* Any rigorous attempt to distinguish reflex from central mechanisms governing temperature regulation presupposes a knowledge of "central" temperature—in this case, the temperature of the blood perfusing the hypothalamus. Analyses in which rectal temperature is regarded as "central" temperature can be correct only (a) if temperatures of rectum and hypothalamus are identical or (b) if these temperatures show identical

cal direction and rate of change under the conditions investigated. The experiments herein reported, employing internal jugular vein or common iliac artery temperature as a first approximation to that of the hypothalamus, fail to substantiate completely either of these assumptions so it suggested that although qualitative generalizations are unlikely to require modification, quantitative conclusions heretofore obtained must be reassessed.

In this study, fine wire (38-42 gauge) copper-constantan thermocouples enclosed in polyvinyl plastic catheters have been introduced for distances of 0.5-20 cm within the lumen of femoral, brachial, radial, and dorsalis pedis arteries, internal jugular, femoral, antecubital, wrist, and ankle veins, and have been left in place for experimental periods of up to 6 hours so that simultaneous determination of as many as 6 intravascular as well as rectal and cutaneous temperatures have been possible. Whereas the more central vascular and the rectal temperature do not differ remarkably under steady state conditions, during periods of environmental change these values deviate significantly from each other, show different time characteristics of their rate of change, and may even, though rarely, change in opposite directions.

**Effects of positive intrapulmonic pressure on muscular contraction.** LEONARD H. ELWELL (by invitation) and JOHN W. BEAN, *Physiology Laboratory, University of Michigan, Ann Arbor*. A continuation of an earlier study by one of us on the effects of increased intrapulmonic pressure on muscular contraction showed that such pressure (260 mm H<sub>2</sub>O for 3 to 5 minute periods) commonly enhanced the response of the Tibialis Anticus Muscle to intermittent single shock stimulation (3 second intervals) applied to the intact or the peripheral end of the sectioned peroneal nerve in anesthetized or decerebrate dogs.

The possibility that this enhancement, gradual in onset and persisting throughout the period of increased pressure, might have been due either to an initial temporary increase in alveolar O<sub>2</sub> tension, or to the subsequent apnea, induced by the pressure was eliminated by appropriate adjustment of the O<sub>2</sub> tension of the compressional air, and by the use of constant artificial ventilation respectively. The volume flow of blood through the leg was markedly diminished and the pH of the arterial blood measured continuously by glass electrode was decreased. Moderately severe hemorrhage which decreases volume flow of blood induced similar enhancement. It is tentatively concluded that the enhancement resulted from an alteration in the removal of metabolites consequent upon the decreased blood flow, and since this enhancement has been missing or less pronounced in direct stimulation of the curarized muscle, it is inferred that the neuromyal function is primarily involved. Sympathectomy in an earlier

study did not eliminate the response to pressure, but the possible involvement of a circulating hormone as adrenalin has not been ruled out.

**The effect of Aminophyllin on cardiac output and renal hemodynamics in man.** DORIS J. W. ESCHER (by invitation), RAYMOND E. WILSON, GEORGE LEINER (by invitation), LOUIS LEITER (by invitation), and SYBIL GOLDAT (by invitation), *Medical Division, Montefiore Hospital, New York 67, N. Y.* CHASIS *et al* (J Clin Invest 17: 683, 1947) observed that in normal men Aminophyllin (Theophylline ethylene diamine) produces an immediate, marked, sustained increase in GFR with a sustained decrease in RPF, after an initial brief period during which RPF also is increased somewhat. Our group has found that in patients with congestive failure Aminophyllin produces a similar immediate increase in GFR with an immediate, but smaller, increase in RPF, which is sustained, unlike that observed initially in normal subjects. To analyze the cardiovascular-renal actions of Aminophyllin, GFR, RPF, and/or cardiac output (by the direct Fick Principle) were determined in human subjects before and after the rapid intravenous administration of Aminophyllin (0.72 grams).

In normal subjects, Aminophyllin generally produces a significant increase in cardiac output, which persists ten to twenty minutes and parallels the brief period of increase in both RPF and GFR, described by Chasis *et al*. The cardiac output, then, returns to normal but the GFR remains elevated for as long as an hour, despite the fall in the RPF to below control levels after the initial renal hyperemia. In chronic congestive failure, as McMichael (Clin Sci 6: 125, 1947) has reported, Aminophyllin produces a marked increase in cardiac output, which is sustained for as long as an hour and probably explains the persistent renal hyperemia observed in these cases. The sustained increase in GFR and filtration fraction observed in both normals and cardiacs probably results, in part, from a direct renal action of Aminophyllin.

**The effect of nicotine on maze learning ability of albino rats.** J. M. ESSENBERG (introduced by GEORGE CLARK), *Department of Anatomy, The Chicago Medical School, Chicago 12, Ill.* Eighteen young, sexually mature albino rats were selected for the experiment. They were males and females from four litters and were treated as such throughout the experiment. Food was withheld from the animals 12 to 18 hours before they were placed in the maze. The Lashley general type of maze was adopted for this work. The first part of the experiment consisted in learning of the maze. The animals were considered ready for nicotine when they could make ten error-less runs for at least two consecutive days. This was followed by daily injections of

one half cubic centimeter nicotine saline solution of 1:1000 or 1:2000, subcutaneously and in some instances intraperitoneally. The maze running began in some instances soon after recovery from the injection, but in most cases, 24 hours later. A record of behavior but particularly the time required and the errors made for 10 runs were kept for each rat. The experiment was designed for 18 to 24 months. Some of the rats were eliminated from the experiment because of lack of intelligence or because of illness. Controls received similar treatment except the nicotine. Of all the animals treated with nicotine, there was a gradual increase in the number of errors per ten runs. As to the time required, a few showed slight differences, but the majority ran high scores, two or three times the control limit.

**Effects of cold on infant rats.** JAMES FAIRFILL (introduced by E. F. ADOLPH). *Dept. of Physiology, The University of Rochester, Rochester, N. Y.* Rats from 0 to 17 days of age were cooled in an atmosphere of oxygen of 2°, 5°, 10°, or 20°C within a glass respirometer surrounded by water. When more than 3 days old, but not before, they manifested large increases in oxygen consumption, which soon diminished as the body temperature fell. In all individuals less than 11 days old, exposed to 3° or lower, the consumption reached zero, it could remain there for 17 hours and still be followed by complete recovery of the animal and acceptance by the mother rat. The rate of heart beat as observed by electrocardiograms slowed, and the beats disappeared at body temperatures between 9° and 1°. These temperatures are below those at which the mature rat's heart stops. When rewarmed in surroundings of 35°, the heart beats, breathing, limb movements and oxygen consumption reappeared within a few minutes. Asystole lasting 13 hours was followed by complete recovery. After two hours of cold exposure, ten percent of the animals failed to recover, their hearts beat irregularly with atypical QRS waves which suggested injury, possibly from anoxia, to the cardiac conduction system. After two hours of exposure all hearts resumed beating during rewarming, subsequent failures of beat occurred only in rats whose body temperatures had been below 8°. Majority survival at 3° body temperature contrasts with adult rats which die at 14° to 16° body temperature. The contrast classes newborn rats as poikilothermic within brief periods of cooling.

**On the mechanism of the action of di-isopropyl fluorophosphate.** EMILY A. FELD (by invitation), MORTIMER A. ROTHENBERG (by invitation) and DAVID NACHMANSOHN. *Department of Neurology, College of Physicians & Surgeons, Columbia University, New York.* The irreversible inhibition of cholinesterase by di-isopropyl fluorophosphate (DFP) requires a measurable period of time dependent upon temperature, DFP concentration,

condition of the enzyme, etc. This feature made it possible to establish a close parallelism in all cases between the rate of irreversible enzyme inactivation and irreversible abolition of conduction. Whatever factor is changed, the two events are influenced in the same way. It has not been possible under any condition to dissociate conduction and cholinesterase activity. Claims to the opposite (Boyarsky, Tobin & Gerard) are based on the use of inadequate techniques. Under the experimental conditions used but determining the enzyme activity with the proper enzyme to substrate ratio, presence of cholinesterase has been demonstrated (Feld, Grundfest, Rotherberg, Nachmansohn, in press). Death of animals following DFP injection coincides with the disappearance of cholinesterase. In surviving animals, cholinesterase is always present, although in a concentration which is on the average about 25 per cent of the initial. The question of enzyme excess and some other aspects of DFP poisoning will be discussed. All findings support the assumption that the toxicity of the compound has to be attributed exclusively to the inactivation of cholinesterase, the enzyme without which the vital process of conduction is impossible.

**Decrease in the radiosodium and thiocyanate spaces during growth.** FRANCIS X. MILLERS (by invitation), and HENRY L. BARNETT (by invitation), HILLY McNAMARA (by invitation) and KENNEDY HARE. *The New York Hospital and Department of Pediatrics, Cornell University Medical College, New York City.* Measured amounts of sodium thiocyanate and  $\text{Na}^{24}\text{Cl}$  were injected intravenously into 10 subjects including premature and full term infants, children and adults. Analyses of serum and urine collected from 1-12 hours later permitted the calculation of the volume of fluid in which these substances were distributed. The results are summarized in the table, in which the available space is expressed in per cent of body weight. Serial ob-

| Weight   | No. subjects | Thiocyanate space<br>(2½-4 hour) | Radiosodium space<br>(2½-4 hour) |
|----------|--------------|----------------------------------|----------------------------------|
| kg       |              | %                                | %                                |
| 2.1-4.5  | 12           | 37.7                             | 42.0                             |
| 4.6-10.0 | 12           | 31.7                             | 34.6                             |
| 10.1-40  | 11           | 29.0                             | 33.4                             |
| 60-75    | 5            | 20.7                             | 26.4                             |

servations were made on 2 babies over a period of 5 months, showing a decrease in the moiety of body weight available as a solvent for thiocyanate and sodium. Simultaneous determinations of thiocyanate,  $\text{Na}^{24}$ , mannitol and inulin spaces indicate that the latter two substances are unsatisfactory for comparison since their spaces never attained a constant value but continued to increase throughout the period of observation.

**Studies on the stability, inhibition and activation of fibrinolytic protease (trypsin plasmin)** JOHN H. FRIGGUSON, and (by invitation) JESSICA H. LEWIS, P. W. BOYLES, B. L. TRAVIS, and E. B. GERHEIM *Dept of Physiology, Univ of North Carolina, Chapel Hill* Employing fibrinolytic and fibrinogenolytic assay methods, data on the relative activity of plasma protease preparations of human, bovine, and canine origin are presented, together with a study of the stability of these materials in dry lyophilized state and in solutions stored at  $-20^{\circ}$ ,  $0^{\circ}$ ,  $28^{\circ}$  and  $37^{\circ}\text{C}$ . Attempts were made to stabilize the activity of these preparations at room temperature using various materials, e.g. acacia, crystalline soybean trypsin inhibitor (SBI), etc. Mixtures of protease and SBI reach optimal fibrinolytic activity at a time much later than that at which untreated enzyme has lost most of its potency. Stability and activity of SBI at various dilutions and the effects of temperature of storage are followed.

The action of SBI is compared with the antiproteolytic activity of natural serum and of the bovine "antifibrinolysin" of L. C. Loomis. Additional experiments on the stability of streptokinase (enzyme activator) are described, together with some data on the stability and interactions of the enzyme precursor.

The extension of these studies to preliminary investigations of antienzyme and proenzyme in normal and pathological sera will be noted.

**The physiological basis for the internal ventilation of clothing** E. S. FETCHER, S. I. RAPAPORT (by invitation) and JOHN F. HALL (by invitation) *The Aero Medical Laboratory, Wright Field, Dayton, Ohio* Neither physiological regulation nor insulating clothing can protect man exposed to a wide range of rapidly changing temperatures, or to very high temperatures. These conditions are of common occurrence in aviation, industry, and the armed services. This problem can be met by replacing the ambient environment by an individual environment created by blowing air under clothing and near the skin. This personal environment can be kept constant over a wide range of ambient temperatures by regulation of ventilating air temperature and flow to compensate for heat leakage through the outer clothing.

Subjects wearing a ventilating harness, and clothing designed for comfort at about  $+50^{\circ}\text{F}$ , have been exposed to  $-30$ ,  $0$ ,  $+70$ ,  $+120$ , and  $+180^{\circ}\text{F}$ . At  $-30$  and  $+180^{\circ}\text{F}$  about 55 CFM (4 l./min) of 120 and  $60^{\circ}\text{F}$  air respectively, were sufficient to maintain thermal equilibrium, and hence comfort, indefinitely. Under proper conditions, the hands and feet require no artificial heating or cooling (see Rapaport, Fetcher, and Hall, this issue). The general relationships between ventilating air temperature and flow, and metabolic rate,

clothing, and environment are defined, the physical and physiological requirements are delineated.

**The influence of substances affecting body temperature on oxygen consumption and glycolysis in brain** J. FIELD, C. N. PEISS (by invitation) and V. E. HALL *Department of Physiology, Stanford University, California* Previous work in this laboratory suggested the hypothesis that when the metabolic rate of the temperature regulating center is increased the center behaves as though its temperature were raised and regulates body temperature at a subnormal level. It thus becomes of interest to examine the influence of substances which raise or lower body temperature, possibly by altering the level at which temperature is regulated (see abstract of Hall, Grant and Field) upon the metabolism of the center. Direct investigation of the metabolism of the center itself has not yet been feasible. However, the available evidence indicates that the course of carbohydrate catabolism is qualitatively similar in the several parts of the central nervous system. Accordingly we have investigated the effect of substances modifying body temperature on the oxidative and glycolytic metabolism of rat cerebral cortex slices which may possibly reflect the metabolic picture in the hypothalamic centers. The influence of graded concentrations of magnesium (as the chloride), typhoid-paratyphoid vaccine (T.P.T.), 2,4 dinitrophenol (DNP) and antipyrine on oxygen consumption and anaerobic glycolysis in rat cerebral cortex at  $37.5^{\circ}\text{C}$  was studied. Conventional manometric methods were

| Substance       | Concentration range | Metabolic effect |   | Effect on body temperature |
|-----------------|---------------------|------------------|---|----------------------------|
|                 |                     | Q <sub>O</sub>   | Q <sub>A</sub> <sup>N<sub>2</sub></sup> |                            |
| Mg mV/l         | 0.15-5.2            | 0                | +                                       | -                          |
| T.P.T.          | 1/50000-1/2000      | 0                | 0                                       | +                          |
| DNP mM/l        | 0.11-13.4           | + to -           | 0                                       | +                          |
| Antipyrine mM/l | 0.054-5.4           | 0                | 0                                       | -                          |

0 denotes mean values same as control + above control - below

used. From this evidence it appears that neither of these over all metabolic processes is directly related to possible change in the set level of the temperature regulating center.

**Urinary tract infections and antibiotics** EDWARD P. FINCH (by invitation) and SEWARD E. OWEN *Veterans Administration, Hines, Illinois* Urines from two hundred and seventy paraplegic patients with urinary tract infections were studied. Bacterial organisms of thirty seven different species were isolated in a series of nearly two thousand culture classifications. Early cases displayed an average of two different organisms while later each case returned an average of five different organism species. The increase is undoubtedly due to the impossibility of sterilizing body parts and to the necessity

for repeated catheterization due to incomplete drainage and incompetent sphincters. Seventy five per cent of the organism types found were gram negative. *Aerogenes* occurred in 62.1% of all cases, *proteus* in 52.8, *pseudomonas* in 43.4, *E. coli* in 40.5, *enterococci* in 39.8, *paracolon* in 33.2, *achromobacter* in 31.4, *streptococci* in 30.7, *staphylococci* in 24.1, *micrococci* in 12.4, *alcaligenes* in 9.1, *diphtheroids* in 8.4, yeasts in 1.8 and three other types in less than 1% of all cases. Separate colonies of the same type organism from the same plate often displayed considerable variation in sensitiveness to penicillin or streptomycin. A more exact prediction of organ sensitiveness to the antibiotics and their probable efficiency in treatment can only be obtained by combining several typical colonies of the same organism and determining the antibiotic resistance of this mixture.

**Effect of magnesium on alkaline phosphatase as influenced by pH, enzyme concentration and aging.** CLARA J. FISCHER (by invitation) and ROY O. GREEP, *Harvard School of Dental Medicine*, Boston, Massachusetts. Purified alkaline phosphatase standing at room temperature loses its activity more rapidly at pH 9.5 than at pH 6.5. The activity can be partly or completely restored when an optimal concentration of magnesium ions is added to the substrate during activity determination. On standing with magnesium phosphatase loses its activity more rapidly regardless of pH and can no longer be activated by adding magnesium to the substrate. Adding magnesium to the substrate during incubation of fresh (highly active) phosphatase produces much less activation than similar treatment of aged samples having reduced activity. This difference can be explained by the assumption that the fresh sample contains a sufficient amount of metal for its complete activation. The activating effect produced by adding an optimal concentration of magnesium to the substrate steadily increases with the duration of standing at pH 6.5 whereas on standing at pH 9.5 there is an initial decline suggesting participation by the recovered inorganic group of phosphatase at this pH. These observations suggest that phosphatase is a metal protein and that the inactivating and reactivating effects involve the specific nature of the metal group of the enzyme protein.

**Changes in zymohexase activity during denervation atrophy of skeletal muscle and their retardation by appropriate electrical treatment.** ERNST FISCHER and RUSSEL V. BOWERS (by invitation), *Baruch Center of Physical Medicine, Medical College of Virginia, Richmond*. Myogen was extracted from mash of normal and denervated rabbit gastrocnemii and purified by dialysis. The zymohexase activity of the crude extract and the purified preparation was determined by the method of Herbert et al. For normal muscle no enzyme activity was

lost by dialysis, while for denervated muscle a loss up to 10% was observed. The amount of protein extractable per gm muscle increases slightly until the second week after denervation, but the enzymatic activity per mg protein declines slowly during the same time. Since the decline of the specific activity nearly balances the increase in extractable protein, and since the small remaining decline in total enzymatic activity calculated per gm muscle can be explained by the relative increase of connective tissue, it is concluded that zymohexase activity is not altered during the first two weeks of denervation. From the third week on, the extractable protein as well as its specific activity diminishes rapidly.

Appropriate electrical treatment has no beneficial effect upon the decline in extractable protein, but diminishes considerably the decline in zymohexase activity per mg protein. In consequence, the total loss in zymohexase activity per gm muscle is about halved by the electrical treatment. Some of the experimental findings indicate that the changes in zymohexase activity are of secondary nature and are rather independent of the wasting of the muscle.

**The lipids of the rat brain in choline deficiency.** PIERO P. FOA and HARRIET R. WEINSTEIN (by invitation), *Department of Physiology and Pharmacology, The Chicago Medical School, 710 S. Wolcott Avenue, Chicago, Illinois*. It has been shown that the lipid composition of the brain is not modified by choline added to an adequate stock diet nor by the amount or the nature of the dietary fat. The only exception to this rule appears to be a slight and temporary decrease in brain phospholipids after protracted cholesterol feeding (References in A. V. Stoesser et al., *Proc. Soc. exp. Biol. and Med.*, 32: 761, 1935). To our knowledge the brain lipids of animals fed a choline deficient diet have not been studied. The following experiment was, therefore, made. Forty male hooded rats (73 to 215 g.) were paired and given the following diet, lard 20, corn oil 20, casein 15, sucrose 20, starch 18, cod liver oil 3, salts 4, plus 1 yeast tablet per day, for 18 to 25 days. One rat in each pair received a diet supplemented with 0.2% choline hydrochloride. The rats were killed by stunning. Brain and liver were immediately removed for analysis. All lipids were determined according to M. H. Hack (*J. Biol. Chem.* 169: 137, 1947) with the exception of cholesterol which was determined according to W. R. Bloor (*J. Biol. Chem.* 24: 227, 1916). The lipid composition of the liver was used as a criterion for choline deficiency. No significant differences were found in the total lipid, total phospholipid, sphingomyelin, acetylphosphatides and cephalin, and cholesterol content of the brain of the choline deficient rats and the pair-fed animals receiving choline.

**Coagulation in plain and partially heparinized**

plasma R H K FOSTER *Department of Pharmacology, St Louis University School of Medicine* The degree of coagulation was visually estimated (J Amer Pharm Assoc 36 243, 1947) on recalcified heparinized or unheparinized citrated beef or sheep plasma (stored in frozen state) The clot was broken up, washed with saline and centrifuged in cellulose nitrate tubes at 18,000 G After compaction into a rubbery deposit it was washed with water, dried and weighed Unheparinized plasma gave clots weighing 4 to 10 mg/ml of plasma Other serum constituents besides fibrin were undoubtedly represented in the weight Centrifugation during coagulation resulted in clots weighing 20% less Clots from unheparinized plasma formed in glass, silicone coated glass or cellulose nitrate tubes were of equal weight At least 50% more heparin was needed to give inhibition in glass tubes comparable to that in the latter two which were about equal in effect Clots from heparinized plasma weighed less and in approximate proportion to the degree of coagulation In the "4+" clotting zone the clot weight decreased very slightly with increasing heparin This was in contrast to the increase in optical density in this region At the point where active visible inhibition commenced both the clot weight and the optical density decreased rapidly Curves of these observations could be approximately but not exactly correlated with the curve based on degree of coagulation because of inherent errors in all three, viz extraneous material in the weighed clot, lack of uniformity of clotting affecting the densimetric measurements and personal errors in judging the degree of coagulation

**Renal and circulatory factors in congestive failure of the circulation** D M FOWELL (by invitation), A P BRIGGS, N C WHEELER (by invitation), J A WINSLOW, JR (by invitation), J W REMINGTON, and W F HAMILTON *Departments of Physiology, Biochemistry, and Medicine, University of Georgia School of Medicine, Augusta, Georgia* A series of patients in various stages of the development and remission of congestive failure is being studied The following determinations are being made cardiac output, intracardiac and intra-arterial pressures, peripheral resistance, circulation times, vital capacity, blood volume, thio-cyanate space, glomerular filtration and sodium clearance Response to bed rest, digitalis, theophylline isopropanolamine (Theopropanol, National Drug Co) and mercurial diuretics are being investigated With clinical improvement there is, as has been observed by others, a reduction of the thio-cyanate space and blood volume, an increase in vital capacity, and in the glomerular filtration and sodium clearance Correlation of these favorable renal and clinical findings with improvement in the circulatory variables studied has not been clearly

established as yet Theopropanol produces a decided, but evanescent, increase in cardiac output with small change in the peripheral resistance Our subjects include patients with renal impairment, and results so far show that the improvement in sodium clearance and the elimination of edema fluid are not associated with any absolute level of glomerular filtration, i e, not with the quantity of sodium presented to the tubules

**Respiratory dead space** WARD S FOWLER (introduced by Julius H Comroe, Jr) *Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia* Respiratory dead space was measured by simultaneous and continuous measurement of volume flow and N<sub>2</sub> content (Lilly-Hervy nitrogen meter) of gas expired following inhalation of 99.6% O<sub>2</sub> The average dead space volume of 30 normal males at rest was 155 cc (19% coefficient of variation), the average expired volume required to wash out the dead space was 117 cc The dead space/tidal volume fraction varies in different individuals Physiological measurements of dead space are affected by 1) *Anatomical volume of the bronchial tree* Maximal variations of inspiratory lung volume changed dead space by 100-150 cc Voluntary hyperventilation and exercise hyperpnea increased dead space equally (50-100 cc) Increase in tidal volume decreased the dead space/tidal volume fraction 2) *Gas diffusion between terminal bronchioles and alveolar spaces* Prolongation of inspiratory time by 1-2 seconds significantly reduced dead space volume, and breathholding during inspiration (20 sec) caused reductions of 45-90 cc 3) *Uniformity of gas mixing throughout the lung* When uneven gas mixing is present, the usual methods of dead space measurement give artificially large results, this method shows directly the degree of uniformity in mixing

**Metabolic balances in the cold environment**  
**II Energy exchanges** JOHN A FRANTZ and JAMES L A ROTH (introduced by J W Heim) *Physiology Branch, Aero Medical Laboratory, Wright Field, Dayton, Ohio* Energy exchange data were obtained on human subjects residing continuously for nine days in the cold environment, -32°C, to appraise the role of a USAF emergency ration in a survival situation Fasting in the same environment for a period of six days provided control data Clothing assemblies and sleeping bags were ade-

|                  | Emergency ration | Fasting |
|------------------|------------------|---------|
| Caloric intake   | 1890             | 0       |
| Caloric output   | 2843             | 2800    |
| Water intake     | 899              | 875     |
| Weight loss, gms | 307              | 891     |
| Body water       | 178              | 493     |
| Body tissue      | 121              | 389     |

quate to maintain normal body temperature and comfortable skin temperatures without the necessity for fatiguing exercise during fasting or subsistence upon the ration. Average daily values are expressed in terms of the standard 70 kg man.

Progressive limitation of voluntary activity was noted in the fasting subjects. The R Q approached 0.72 with continued fasting, especially for activities requiring more rapid caloric expenditure. The metabolic cost for a standard work load on the treadmill was increased during low temperature exposure. This was not observed during the activities in which the hobbling effect of the clothing did not participate, i.e. lying, sitting and standing quietly. Considering ability to perform psychomotor tasks, gross work capacity, and maintenance of body comfort in the fasting and non-fasting subjects, food appears to be of secondary importance to the adequacy of clothing and sleeping bag for survival in low ambient temperatures.

**Correlation between signs of toxicity and cholinesterase level of brain and blood during recovery from di-isopropyl fluorophosphate (DFP) poisoning.** A M. FREEDMAN, (by invitation) and H. E. HIMWICH, *Army Chemical Center, Medical Division, Edgewood, Maryland*. Male rats were injected with Di-Isopropyl Fluorophosphate (DFP) (2.0 mg/kgm subcutaneously) and sacrificed at intervals varying from one hour to 14 days, at which time the rats were asymptomatic. Prior to sacrifice, signs of toxicity were carefully noted, then brain, plasma, and erythrocyte cholinesterase activity was determined. Plasma cholinesterase rapidly returns to normal in a few days. Erythrocyte cholinesterase remains at zero for about 48 hours and then rapidly regenerates. Brain cholinesterase increases more than three fold in the first 24 hours and thereafter the rate of regeneration decreases. The clinical signs of DFP toxicity diminish as the brain cholinesterase rises. The level of brain cholinesterase corresponding to the various degrees of severity of clinical signs in this recovery period is in general similar to that which has recently been demonstrated for the acute phase.

These data suggest again that the severity of the signs are related to the brain cholinesterase activity. The correlation between cholinesterase level of brain and blood that has been demonstrated immediately after DFP injection is not found here. Further, an explanation is afforded to the findings of Harvey et al. that although there is a correlation in the human between red blood cell cholinesterase and symptoms immediately following DFP injection, when the drug is withdrawn symptoms disappear while the RBC cholinesterase remains low. This phenomenon appears to be due to the very rapid rate of regeneration of brain cholin-

esterase as compared to RBC cholinesterase in the first day or two after the injection.

**The effect of size, sex and pregnancy on the lethality of di-isopropyl fluorophosphate (DFP).** A. M. FREEDMAN, (by invitation) and H. E. HIMWICH, *Army Chemical Center, Medical Division, Edgewood, Maryland*. Previous work has revealed that newborn rats are much more sensitive to a dose of 2.0 mg/kgm of Di-Isopropyl Fluorophosphate (DFP) subcutaneously than adult rats of both sexes weighing between 100-200 gm. In the course of experiments, it was observed that pregnant females were also particularly susceptible to the toxic effects of DFP. In order to study the importance of size and sex in the sensitivity of the pregnant female, the following preliminary mortalities were found in various groups of rats.

| Rats                          | No | Died | % Died |
|-------------------------------|----|------|--------|
| Males (100-200 gm)            | 20 | 6    | 30     |
| Females (100-200 gm)          | 20 | 3    | 15     |
| Males (250-400 gm)            | 20 | 12   | 65     |
| Females (250-400 gm)          | 10 | 6    | 60     |
| Pregnant females (200-400 gm) | 18 | 17   | 94     |

Thus, there is no significant statistical difference between normal males and females of the same weight. However, there is a significant difference in the susceptibility between light and heavy rats and between the latter and pregnant females of similar weight. The mechanism of this variation in susceptibility is being investigated.

**Conduction of painful impulses from the extremities via the sympathetic nervous system.** L. W. FRIEDMAN, H. B. SHUMACKER, JR. (by invitation), E. E. WATSON (by invitation) and N. M. STAHL (by invitation), *The Department of Surgery, Yale University School of Medicine, New Haven, Connecticut*. Sympathetic denervation of the extremities often provides relief of pain in patients suffering from various vascular disorders and regularly in patients with major causalgia. When pain associated with ischemia is thus relieved in limbs, the circulation of which is improved by the procedure, one can explain the relief by section of efferent sympathetic fibers. When pain is relieved in such cases without improvement in circulation and in cases of causalgia, it is difficult to understand the mechanism on any basis other than interruption of afferent pain fibers.

Studies on dogs utilizing various responses as evidence of pain suggest that there are multiple pathways by which painful impulses arising in the lower extremities may reach consciousness. Section of the spinal cord at sufficiently high levels, anterior and posterior rhizotomy of sufficient extent, and complete bilateral sympathectomy appear to eliminate responses to acute vascular injuries. Utilizing the local vasoconstrictor response to

acute chemical injury of the femoral vein as an index of pain, experiments would indicate that afferent pain fibers are present in the sympathetic nerves, interruption of which serves as a satisfactory explanation for relief of pain.

**A comparative study of the effects of several anticholinesterases on central nervous integration** JEANE SISKELE FREY (by invitation) and ROBERT GELSELL *Physiology Laboratory, University of Michigan, Ann Arbor* All anticholinesterases studied produced coordinated hyperpnea. Carbon dioxide was most dependable, next DFP, physostigmine, X-substance (courtesy Col Wood), and prostigmine. Larger injections produced uncoordinated hyperpneic activity with total ventilation diminished rather than increased. Torsal movements were greatly diminished while facial accessory respiratory contractions were simultaneously augmented. Whereas diminished torsal activity suggests lack of respiratory stimulation, intensified facial activity suggests powerful respiratory stimulation. It is consequently concluded all experiments gave evidence for central potentiation of nervous activity. Atropin given during the coordinated hyperpnea produced by anticholinesterase poisoning reduced all respiratory movements. In the more severe poisoning where torsal movements were diminished and facial movements augmented atropin simultaneously restored torsal movements and diminished facial movements. Atropin simultaneously augmented inspiratory and reduced expiratory components of torsal breathing. Anticholinesterases produced comparable differential effects on respiratory components.

Our results suggest that the principle of bi-polar function of neurons combined with reciprocal inhibitory interlocking of half-centers offers reconciliation of confusing differences of opinion on the theory of central humoral integration. In accordance with this working hypothesis four strategical regions are exposed to differential anticholinesterase activity—the excitatory and inhibitory poles, respectively, of antagonistic neurons. Coordinated hypercapnic hyperpnea could accordingly be attributed to symmetrical effects of increased physiological anticholinesterase activity. The uncoordinated hyperpnea produced by foreign anticholinesterases could similarly be assigned to a differentially increased anticholinesterase activity at the four cardinal regions of reciprocally coupled neurons. Imbalance of potentiation is regarded as an essential factor in the interpretation of anticholinesterase activity.

**A comparison of the renal clearances of allantoin and inulin in man** M. FRIEDMAN and S. O. BYERS (by invitation) *The Harold Brunn Institute for Cardiovascular Research, Mt Zion Hospital, San Francisco* A preliminary study (Proc

Soc Exp Biol and Med, to be published) indicated that when six human subjects were given allantoin by mouth, the latter's renal clearance was 123 cc per minute. This suggested that the clearance of allantoin might be equivalent to that of inulin and as such, a measure also of the rate of glomerular filtration. Simultaneous allantoin and inulin clearances were performed on five normal males. Allantoin was administered by mouth and inulin by continuous intravenous infusion. The average allantoin clearance of these five subjects was 114 cc per minute (Range 96 to 123 cc). The average inulin clearance was 108 cc per minute (Range 98 to 118 cc). The allantoin clearance/inulin clearance ratio was 1.05 (Range 0.98 to 1.17). In view of these results, it would appear that the renal clearance of allantoin may be used as a measure of the rate of glomerular filtration in man.

**Treatment of non-specific ulcerative colitis for one year with intestinal extracts** M. H. F. FRIEDMAN, B. F. HASKELL (by invitation), and J. M. WALDRON (by invitation) *Department of Physiology, Jefferson Medical College, and Department of Surgery, Jefferson Hospital, Philadelphia* Previously (Federation Proceedings, 6, 107, 1947) we reported that oral administration of extracts of hog's small intestine mucosa appeared to exert a beneficial effect in patients with non-specific ulcerative colitis. Treatment was based on the hypothesis that the disease resulted from a deficiency of an intrinsic protective factor normally present in the intestinal mucosa. Of 52 patients now under treatment 23 have been followed for a period of over one year. Only 2 of these 23 patients have failed to respond favorably to intestinal extracts. Improvement, commencing in 3 to 5 weeks, consisted of reduction in frequency of bowel movements, increase in stool consistency and disappearance of gross blood and mucus. Sigmoidoscopic evidence of mucosal healing was present usually after symptomatic improvement was noted. The lower rectal mucosa usually was last to heal. In cases with extensive involvement of the colon of long standing mucosal healing and symptomatic improvement were noted but radiologic examination did not show reversal of fibrotic changes in the colon structure. Withdrawal of intestinal extracts or substitution of a placebo were followed by relapse with remission occurring on resumption of treatment with intestinal extracts.

Isolation of the substance in the intestinal extract which is responsible for the beneficial effects in ulcerative colitis is greatly hindered by lack of an assay procedure using animals with similar lesions of the colon induced experimentally.

**Preparation and assay of secretin** M. H. F. FRIEDMAN, and J. E. THOMAS *Department of Physiology, Jefferson Medical College, Philadelphia* In the method to be described, separation



of secretin from inert substances is achieved easily in the fractionation procedures. Neither vacuum distillation nor adjustment of pH to maximum solubility or precipitation points is involved. Reproducible results by different workers in two separate laboratories have been obtained. The secretin prepared by this method is easily freed from contamination by vasodepressor substances, pyrogens, cholecystokinin, enterocrinin, and pancreozymin. Preparations with a potency of 64 to 160 Agren cat units (16 cat units equals 1 dog threshold or 1 clinical unit) per milligram, assayed against a standard reference secretin by a procedure to be described, has been given intravenously to over 150 human subjects without any demonstrable reactions and has been found to be non-antigenic in both animals and man.

**Renal function related to increased intra-abdominal pressure in anesthetized and unanesthetized dogs.** DAVID M. FRENCH, PEDRO A. MOLANO and WALTER M. BOOKER (introduced by A. B. Luckhardt) *Dept. of Pharmacology, Howard University School of Medicine, Washington, D. C.* We have reported circulatory and respiratory effects brought about by acutely increasing the intra abdominal pressure in anesthetized dogs, and effects on the urinary output and the non-protein nitrogen of the blood in chronic animals (unanesthetized) whose intra abdominal pressure was increased gradually from week to week. No clearance studies were mentioned in these reports. In view of the marked increase in venous pressure in the abdomen and below in acute experiments and the evidence of kidney involvement in the chronic experiments (decreased urinary output, albuminuria, passive congestion demonstrated at necropsy), it was thought worthwhile to attempt to correlate the clearance efficiency of the kidney with various levels of intra-abdominal pressure, both in acute anesthetized (pentothal) and chronic unanesthetized animals. After urine and blood samples were collected, the latter from the femoral vein, 10 mgm. of creatinine was injected every 15 minutes in some instances, or every 10 minutes in others. Blood and urine samples were drawn at the same intervals. After a control period the intra-abdominal pressure was increased to 10, 20, 30, 40, and 50 mm. of mercury for half hour periods. We have observed that at low intra-abdominal pressure (10 mm. or less) there is no effect on the clearance of creatinine. At moderate intra abdominal pressures (20 to 30 mm.), there is a gradual reduction in the clearance of creatinine and at higher levels, the clearance falls off to very low levels. In the chronic experiments the clearance of phenol-sulphonphthalein and of creatinine is dependent to some degree upon the speed with which the intra abdominal pressure is raised from week to week. Evidence of adjustment to given pressures can be seen, pro-

vided increases in intra abdominal pressure are not too rapid. At high intra abdominal pressures (40 to 50 mm.), however, phenol-sulphonphthalein and creatinine are significantly reduced in clearance rate.

**The effect of rutin in experimental frostbite.** FREDERICK A. FUHRMAN *Department of Physiology, Stanford University School of Medicine, Stanford, California.* The extent of tissue loss and course of injury following freezing were studied in the feet of rabbits given the flavonol glycoside, rutin, and the results were compared with those obtained on control animals (*J. Clin. Invest.* 26: 229). Closely clipped hind feet of anesthetized rabbits were frozen for 3 minutes at  $-55^{\circ}\text{C}$ . This severity of injury in 15 control animals invariably resulted in gangrene of the entire injured region, 11 animals subsequently lost all the frozen part, while 4 retained a narrow tongue of tissue on the plantar surface. Rutin was administered to 10 rabbits by stomach tube in doses of 50 or 100 mgm./kgm. daily. Six animals were pretreated with rutin for 1 to 10 days before frostbite, while 4 received rutin immediately following injury. The time required for development of wet gangrene was significantly longer in the rutin-treated animals than in the controls. In none of the rutin-treated animals was the extent of tissue loss as great as in control animals, and in most cases it was confined to the toes. Rutin delayed the loss of intravenously administered trypan blue into frosted areas of abdominal skin of rats. Recent results indicate that rutin in propylene glycol (50 mgm./kgm. i.v.) lowers the threshold concentration of epinephrine necessary to produce closure of capillaries in the rat mesoappendix. It seems probable that the effect of rutin in frostbite may be the result of an alteration in the pattern of local blood flow.

**Effect of anoxia on contractility and metabolism of intestinal smooth muscle.** R. F. GURCHCOTT (by invitation) and EPHRAIM SHORR *Dept. of Medicine, Cornell University Medical College and The New York Hospital, New York City.* Contractions of segments of rabbit duodenum and jejunum were recorded with an isotonic lever. The medium at  $37.5^{\circ}$  was Krebs bicarbonate 200 mg. % glucose. With 95%  $\text{O}_2$ -5%  $\text{CO}_2$  bubbling through the medium, the tone, amplitude and frequency remained relatively constant for as long as 5 hours. On substituting 95%  $\text{N}_2$ -5%  $\text{CO}_2$  after a control aerobic period, tone decreased markedly and contractions became sporadic and of irregular frequency and amplitude. On restoration of aerobic conditions, recovery was manifested by return of tone to or above that of the control period, and restoration of frequency and amplitude. Failure of recovery was indicated by absence of increase in tone above the anaerobic level, and failure to resume the aerobic contraction pattern. On the basis of these criteria,

intestinal segments exhibited good recovery after anaerobiosis lasting up to 120 minutes. Data will be presented indicating that glycolysis provides energy for the sporadic contractions and maintenance of viability during anaerobiosis. When intestinal segments were suspended in glucose-free medium and first allowed to deplete their substrate stores (Furchgott and Shorr, *Proc Soc Exp Med Biol*, **61** 280 (1946)), they gave no contractions during a subsequent 60 minute anaerobic period, and usually failed to recover on the reintroduction of oxygen and glucose. The contractility studies with rabbit intestine agree well with metabolic experiments with strips of smooth muscle from dog small intestine. After 60-120 minutes of anoxia, dog smooth muscle showed good recovery of  $Q_{O_2}$  and high-energy phosphate stores on being returned to aerobic conditions.

**Determination of the etiology of pathological effects of explosive decompression on the lungs of rats** MADELINE FUSCO, (by invitation), HENRY MELLFORTE, (by invitation) and FRED A. HITCHCOCK. *The Laboratory of Aviation Physiology, The Ohio State University, Columbus*. In order to determine the etiology of the pathological effects of explosive decompression on lungs of rats, rats were Group A—Explosively decompressed to 30 mm Hg under an experimental schedule allowing 80% survival, Group B—Exposed to 97% nitrogen at ground level of such duration to allow 80% survival, Group C—Sacrificed without experimental procedure. The lung volumes of these rats were determined as described in previous publications. Grossly, the anoxia alone produced some edema and hemorrhage of the lung but this effect was much greater in rats explosively decompressed. Measurements of lung volume, lung weight, and lung air indicate that only part of the effects of explosive decompression on the lungs are due to anoxia. Studies are in progress to determine the actual degree of edema present and the composition of the edema fluid.

**Peripheral vasodilator effect of a substance present in normal human urine** CLIFFORD G. GADDY (by invitation), HAROLD D. GREEN and J. MAXWELL LITTLE. *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina*. The intravenous injection of urine into dogs causes an increase in cardiac output, a decrease in peripheral resistance and a decline in mean arterial pressure (Green, Little and Hester, *Fed Proc* **6** 114, 1947). In the present study, injections of urine were made into the femoral artery of heparinized dogs anesthetized with sodium pentobarbital. Venous outflow was recorded by a modified Gaddum technique (*J Physiol* **LXVII** *Proc Physiol* **XVI**). Collateral venous return was prevented by a tourniquet. A constant mean ar-

terial pressure was maintained with a "Lampson" pressure regulating bottle. Urine, collected and handled sterilely, was dialyzed in "Viskos" bags at 9°C and stored at -20°C. Representative samples of urine were found pyrogen-free using the rabbit as test animal. All injections were 0.2 cc of the urine preparation or dialyzed saline in 1.0 cc of blood given in 20 seconds. The response was always noted with 20 seconds after injection started and lasted 60-100 seconds. Effects are expressed as per cent changes in total flow. Results: Plain unaltered urine +41.5 to +131.6 (mean +62.7%), Seitz filtered urine -24.1 to +132.8 (mean +58.3%), urine dialyzed 24 hours in tap water and 24 hours in distilled water +3.5 to +100.4 (mean +56.0%), urine, dialyzed after Seitz filtration +30.7 to +104.5 (mean 64.4%), dialyzed saline +10.6 to +34.8 (mean +32.6%). The peripherally acting vasodilator substance found in normal human urine is partly removed from urine by Seitz filtration, is not dialyzable and is not the result of bacterial contamination.

**Exchange of carbon dioxide and oxygen in the nasopharyngeal part of the respiratory dead space** MORTON GALDSTON and SEYMOUR A. HORWITZ (introduced by J. Murray Steele). *Department of Medicine, New York University College of Medicine and Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, New York 17, N. Y.* The exchange of carbon dioxide and oxygen in the nasopharynx was studied in two healthy adults at rest in a recumbent posture. Air samples were drawn at the end of inspiration through a small rubber catheter into an evacuated mercury gas sample tube. Precautions were taken to prevent contamination of the samples with room air and previously expired air. In 19 samples of pharyngeal air collected within 2 seconds after the end of inspiration there was an average rise of 2.8 mm Hg  $CO_2$  and fall of 2.6 mm Hg  $O_2$  as compared with inspired room air which contained 20.93 volumes per cent oxygen and 0.03 volumes per cent carbon dioxide. These values correspond to approximately 7 per cent of the carbon dioxide and 5 per cent of the oxygen tension change commonly believed to occur only in the alveoli. During breath-holding there was a progressive increase in  $pCO_2$  and a generally slower decrease in  $pO_2$ . A prompt fall in the rate of gaseous exchange occurred after voluntary hyperventilation. There is reason to believe that the gas exchange occurs across the mixed glandular secretions which bathe the pharynx.

The results of these studies have a direct bearing upon such related physiological problems as the size of the pulmonary dead space based on carbon dioxide as compared with oxygen exchange, ratio of carbon dioxide production to oxygen consumption

(RQ) based on analyses of expired air and alveolar air, and the indirect method of calculating the level of mean effective alveolar air pO<sub>2</sub>.

**Studies of the pathological physiology of negative "G" in animals and man** J L GAMBLE (by invitation), R S SHAW (by invitation), O GAUER (by invitation) and J P HENRY *Aero Medical Laby, Air Materiel Command, Wright Field, Dayton, Ohio* Increased pressure in the veins of the head from negative acceleration causes congestion and eventually petechial hemorrhages in the conjunctivae and in the mucous membranes of the accessory sinuses and middle ear. The vessels of the brain, surrounded by incompressible media in the "closed box" of the skull, do not rupture from short exposures. However, two subdural hematomas were observed in ten animals given four two-minute exposures to accelerations of negative 7 g repeated at short intervals. The increased venous pressure also causes edema in the cephalad portions of the body with a retrobulbar edema which may cause diplopia in humans. If the exposure is prolonged for more than five seconds, evidences of heart and central nervous system disturbance are seen. Electrocardiograms usually show bradycardia in both animals and humans. All degrees of heart block and various types of ectopic rhythms occurred in the dogs and interstitial myocardial hemorrhages were occasionally demonstrated. Blood pressure recordings from animals and humans manifesting these "vagal effects" show a fall in arterial and a rise in venous pressure causing a reduction in the arterio-venous differential pressure. Rabbit electroencephalograms following exposures to negative g showed large slow waves and a depression of the normal activity. Four dogs died with respiratory failure out of the ten given four two-minute exposures to negative 7 g.

**Effect of satiation on the intensity of the conditional and unconditional salivary secretion** W HORSLEY GANTT *Pavlovian Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins Hospital* The salivary conditional reflex (cr) depends upon many factors—kind of food, nature and intensity of conditional stimulus (cs) (Pavlov) and exponentially upon the amount of food—unconditional stimulus (Gantt). These definite relationships have been obtained under strictly controlled conditions—in a hungry animal. The following experiments were designed to show the effect of the state of hunger on the crs.

Dogs having stable secretory crs measured through a parotid fistula were used. After a daily series of readings of the salivary cr the dog was fed to satiation of food to which he was conditioned—500 to 2000 gms. After satiation the animal was negativistic toward food, and the cr fell immediately to zero. After some minutes it would eat a little food but the cr remained nearly zero. The

UR showed no constant deviation. In four dogs (Kompa, Billy, Pat, Sechs) the cr before satiation averaged 0.666 cc, after satiation 0.09 cc (30 second readings), the UR 0.80 and 0.71 cc respectively. The laws for cr intensity are seen only under rigidly controlled conditions, the most powerful factor is not intensity and kind of stimulus (cs or US), but the underlying emotional state of the animal—as the emotional tension decreases so does the conditional response, which may change within a few minutes from maximum to zero. The UR, however, maintains its level in spite of the changing emotional state. There is thus a sharp cleavage between cr and its corresponding UR.

**Comparison of the effect of some steroids on the hearts of rats with choline deficiency** JOSEPH H GAST and HAROLD L DONSON (introduced by Allen D Keller) *Dept of Biochemistry, Baylor Univ College of Medicine, Houston, Texas* Rat hearts were studied before and after 28 days administration of five representative steroids by planimeter measurements of the frontal cardiac area (FCA) from duplicate roentgenograms, leads II and III of the electrocardiogram and measurements of the blood pressure in the tail. Terminally the actual heart weight was compared to that calculated from final body weight by the equation of Ryland as used in previous studies (Fed Proc 4, 22, 1915). All animals with normal FCA initially showed no changes, a few with larger initial FCA showed decreases after injection of female sex hormones. Electrocardiographic studies showed heart rates to vary widely, but there was no axis deviation, and no change in PR interval, QRS, or in T waves, though P waves were found to be extremely labile. Contrary to some investigators no elevation in blood pressure was observed, however, any neurogenic effect may have been abolished by the pentobarbital used in all manipulations. Heart weights were uniformly low in all series. Microscopic examination of sections of adrenals, gonads, aorta, kidney, liver and hearts demonstrated only the expected changes in the liver and gonads, except for non-specific fatty infiltration in the adrenal medulla of 7 of the animals.

**Explosive decompression of monkeys at extreme altitudes** SAMUEL GELFAN and GEORGE D DAVIS (by invitation) *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn* The work on explosive decompression of rats as reported at the last meeting has been extended to monkeys. In addition to EKG and respiration, continuous recording was also maintained of blood pressure and EEG. The systolic and diastolic blood pressures were registered through an indwelling arterial needle connected with a Statham strain gauge pressure transmitter and recorded simultaneously with the other variables on a Grass electroencephalograph. As in the case of rats,

explosive decompression per se even up to 75,000 feet altitude does not kill the monkeys. When recompressed at free fall rate, the animals have survived explosive decompression to such altitudes in oxygen and at least 60,000 feet in air.

**Studies of arterial oxygen saturation in patients with suspected arterial hypoxemia, with use of a modified oximeter.** J. E. GERACI (by invitation), GEORGE E. MONTGOMERY, JR. (by invitation) and EARL H. WOOD. *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota.* Resting arterial oxygen saturation in 27 patients with suspected arterial hypoxemia has been measured by Van Slyke analysis of samples of arterial blood and by photo-electric measurements (see abstract, Wood and Geraci) made on the ear of the patient at the time of withdrawal of the sample. The average resting arterial oxygen saturation as determined by Van Slyke analysis was 79.1 (13.4 to 98.2) per cent. The average difference between the photoelectrically determined arterial oxygen saturation and the results of Van Slyke analysis was 0.9 (-5.2 to 5.5) percentage points. Ninety-three per cent of the differences were within  $\pm 5$  percentage saturation points. Multiple blood samples were obtained (see abstract, Wood, Montgomery, Geraci) from ten patients with cyanotic congenital heart disease. The average changes in arterial saturation in percentage points as measured photoelectrically and by Van Slyke analysis were -4.0 and -3.3 after five minutes in the erect posture, -19.2 and -18.4 after walking two to five minutes at 1.7 miles per hour, and +9.2 and +9.5, respectively, after breathing 100 per cent oxygen for ten minutes. In a comparable series of eight patients, with the use of the Millikan CMR model 13 compensated circuit oximeter, the average decrease in saturation with exercise was 13.4 percentage points, as compared to the average decrease of 19.6 percentage points determined by Van Slyke analyses. Decreases in arterial saturation measured by the Millikan oximeter averaged 6.2 (+2 to -21) percentage points less than the results of Van Slyke analyses. In the modified oximeter group, this average difference was 1.4 (-2.9 to 4.9) percentage points. Figures in parentheses are extreme values.

**Staphylocoagulase and staphylokinase.** E. B. GERHEIN (by invitation), J. H. FERGUSON, and B. L. TRAVIS (by invitation). *Dept. of Physiology, Univ. of North Carolina, Chapel Hill.* It has long been known that Staphylococci particularly pathogenic strains, can coagulate ovalated or citrated plasmas and certain fibrinogen preparations. Smith and Hale (1944) showed the necessity for a cofactor in the coagulase reaction. The present study confirms the need for such cofactor and identifies it in a number of materials, especially the albumin fraction of plasma or serum. The staphylococcal

agent, which we propose to call *prostaphylocoagulase*, is precipitated by established methods from chilled broth culture Staph. aureus filtrate, or centrifugate, by 3 vols. of cold 95 per cent alcohol. Its precursor nature is shown by failure to cause coagulation when added to bovine Plasma Fraction I (Armour's), whereas the fibrinogen is clotted to typical fibrin (dark-field microscopy) when cofactor is also supplied in the form of human serum albumin. In this system, lacking the precursor of the fibrinolytic protease (trypsin of plasmin), there is no clot lysis in 10 days at 37°C. However, human Plasma Fraction I (Harvard), previously shown to contain trypsinogen (protease precursor), and currently found to have the coagulase cofactor as well, is first clotted with prostaphylocoagulase and subsequently shows fibrinolysis. Thus, the staphylococcal product acts in a similar manner to streptokinase, in the lysis phenomenon only, although typically weaker. The term staphylokinase is appropriate for the factor responsible for this action.

**The immediate pressor effect of desoxycorticosterone acetate in hypertensive and normotensive subjects.** MELVIN L. GOLDMAN (by invitation) and HENRI A. SCHROEDER. *Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri.* Attempts were made to investigate the immediate effects in man or the intravenous injection of certain steroid compounds on the arterial pressure. Blood pressure was measured by a Hamilton optical manometer, blood content of the ear was estimated by a photoelectric plethysmograph, and cardiac output by the ballistocardiogram. The intravenous injection of 5 mg. of desoxycorticosterone acetate (dissolved in 2.5 cc. of propylene glycol) was followed by significant elevations of the blood pressure in hypertensive individuals. The responses observed lasted up to thirty minutes after injection. Similar effects did not occur in most normal individuals. Cardiac output, venous pressure, blood content of the ear, or electrocardiograms were not altered. The effects of other steroid compounds are being studied, among them progesterone was found to have a similar, although less powerful, action. From these results it would appear that desoxycorticosterone acetate can act as a pressor substance in some hypertensive individuals.

**Calculation of effective afferent and efferent renal resistance.** DOMINGO M. GOMEZ (introduced by H. W. Smith). *New York University College of Medicine.* Differential equations have been presented (La Revue Scientifique, No. 3272, May 1, 1947) defining hydrostatic pressure, blood flow and oncotic pressure in the kidney. The application of these equations to renal data involves knowledge of the locus at which filtration equilibrium is

reached. When capillary resistance is great, equilibrium is reached in the glomeruli, when it is small equilibrium may not be reached until the peritubular capillaries. The actual locus of equilibrium is unknown. Assuming that filtration equilibrium is reached in the efferent arteriole the following approximate relations may be drawn

$$(1) \quad R^{(a)} = \frac{P_m - h_o' - H_o}{Q_o} \\ = \frac{(R_a + b) \left( \frac{1}{E_G} + \frac{1}{E_R} \right) + R_a R_E}{\frac{1}{E_G} + \frac{1}{E_R} + R_E}$$

$$(2) \quad R^{(E)} = \frac{h_o + H_o - P_v}{Q_o} \\ = \frac{(R_E - b + R_z) \left( \frac{1}{E_G} + \frac{1}{E_R} \right) + R_z R_E}{\frac{1}{E_G} + \frac{1}{E_R} + R_E}$$

$$(3) \quad F_t = \frac{q_o'}{q_o} = \frac{R_E}{\left( \frac{1}{E_G} + \frac{1}{E_R} + R_E \right) (1 - C_o)}$$

where  $R^{(a)}$  is the total resistance between the aorta and the locus of filtration equilibrium, and  $R^{(E)}$  the resistance beyond this locus,  $P_m$ , mean arterial pressure,  $H_o$ , capsular pressure,  $Q_o$ , whole blood flow,  $h_o'$ , oncotic pressure at equilibrium,  $C_o$ , hematocrit,  $R_a$ ,  $R_E$  and  $R_z$  the resistances of the afferent arteriole, efferent arteriole and collecting venule, respectively,  $E_G$  and  $E_R$ , permeability coefficients in the glomeruli and in the peritubular capillaries,  $P_v$ , venous pressure,  $b$ , locus of equilibrium relative to the efferent arteriolar resistance,  $q_o'$ , filtration rate,  $q_o$  afferent plasma flow,  $F_t$ , filtration fraction. Since  $R^{(a)}$  and  $R^{(E)}$  are series-parallel resistances proximal or distal, respectively, to the point of equilibrium, we designate them as effective afferent and efferent resistance.

**The in vitro metabolism of testosterone to  $\Delta^4$ -androstenedione-3,17 and cis-testosterone by rabbit liver homogenate.** JOSE GONGORA (by invitation) and CHARLES D. KOCHAKIAN, *Department of Physiology and Vital Economics, University of Rochester, Rochester, New York*. In previous reports from this laboratory it has been demonstrated that testosterone is converted by rabbit liver slices to  $\Delta^4$ -androstenedione-3,17 and cis-testosterone. It has been demonstrated now in three separate experiments that these changes also occur when the testosterone is incubated with a homogenate of rabbit liver prepared in a Waring

blender. The products isolated accounted for 92 per cent of the original testosterone. The recovered material consisted of 87 per cent testosterone, 11 per cent  $\Delta^4$ -androstenedione-3,17 and 2 per cent cis-testosterone.

**The relation of the endocrine gland system to macrophagic activity.** ALBERT S. GORDON and GRACE F. KATSH (by invitation), *Department of Biology, Washington Square College of Arts and Sciences, New York University*. In a previous communication, one of us (A. S. G., Fed. Proc. 5, 1946) reported a diminution in phagocytic activity of splenic macrophagic tissue following adrenalectomy in rats. In addition, administration of adrenal cortical extract, but not DCA, resulted in greater uptake of the colloidal agent (thorotrast). In the present work, 19 normal, 37 hypophysectomized and 7 starved rats were injected intravenously with 0.3 cc thorotrast and killed 20 hours later. The spleens were then analyzed for thorium uptake. Seven of the hypophysectomized rats received 7 cc of an adrenal cortical concentrate over a 2 day period prior to thorotrast administration, one cc of cortical extract was given 9 hours after the injection of thorotrast. Six of the hypophysectomized rats received similar quantities of 1% saline solution. The remaining 24 hypophysectomized rats were untreated. The 7 starved rats were restricted to a 5 gram diet of normal laboratory ration/day over a 32 day period which resulted in a 30% body weight loss. The mean values  $\pm$  standard errors for thorium uptake/gm dried spleen were the following: 1) normal untreated—43.3  $\pm$  3.7 mg; 2) hypophysectomized, untreated—51.2  $\pm$  6.5 mg; 3) hypophysectomized + saline—38.1  $\pm$  9.3 mg; 4) hypophysectomized + cortical extract—146.2  $\pm$  14.9 mg; 5) normal starved—107.9  $\pm$  12.7 mg. The thorium was observed to be localized largely in the macrophagic tissue.

Thus although hypophysectomy does not significantly affect phagocytosis by splenic macrophagic tissue, administration of cortical extract will greatly augment this activity. The question as to whether starvation, in increasing phagocytosis, acts through the adrenal cortex is being studied.

**Acute hypothermia in guinea pigs.** R. E. GOSSELIN (introduced by E. F. Adolph), *Dept. of Physiology, The University of Rochester, Rochester, N. Y.* Severe reductions of body temperature were produced in 30 unanesthetized mature guinea pigs by partial immersion in ice water. Measurements included colonic temperature, electrocardiograms, ventilation rate (minute respiratory volume), oxygen consumption, and  $CO_2$  production. Regardless of the rate of cooling or rewarming, heart rates varied linearly with colonic temperature between 23° and 35°C, falling 18 beats per minute for each decrement of one Centigrade degree.

Below 23°C the drop was erratic and often precipitous. Premature beats of auricular and ventricular origins, auriculo ventricular and intraventricular blocks, and reversible ventricular fibrillation were all noted.

The early phases of cooling were characterized by increased struggling, hyperpnea, enhanced metabolism and cardiac slowing. When cooling at 0.2 to 0.3 degree per minute, maximal ventilation occurred at 35°C (33 to 37°) and averaged 180% of pre-immersion values. Maximal rates of oxygen consumption then averaged 170% of pre immersion rates, or 250% of estimated basal values. Below 33°C, ventilation and oxygen consumption diminished progressively with temperature. Relative to the oxygen uptake, however, the ventilation rate remained high to the stage of complete apnea. Therefore the progressive fall in oxygen consumption cannot be ascribed to failure of external respiration.

Some animals recovered after the rectal temperatures were as low as 18°, others succumbing at temperatures as high as 22°. This large variation in lethal temperature may be due to uneven distribution of temperature. The mechanisms of hypothermic death remain obscure, but these data suggest that severe circulatory inadequacy often precedes respiratory failure.

**Oxygen in bone marrow blood during prolonged hemorrhagic anemia.** WILSON C. GRANT (introduced by Walter S. Root) *Department of Physiology, College of Physicians and Surgeons, Columbia University.* The purpose of this study was to observe directly the O<sub>2</sub> saturation of blood in red bone marrow during a period of constant and severe erythropoietic stimulation, after all reservoirs of preformed erythrocytes had been depleted. Adult dogs on stock diet were rendered anemic by repeated small hemorrhages. In 10 to 15 days this procedure lowered the Hb to 6 to 8.2 gm per cent (O<sub>2</sub> capacity) where it was then maintained for 40 to 60 days by the removal of blood every 2 to 3 days. O<sub>2</sub> saturation measurements were made on arterial and jugular venous blood and on marrow blood obtained from the humerus. Control observations were collected during one to two weeks prior to bleeding. The determinations were resumed at weekly intervals 10-15 days after the start of the hemorrhages when the Hb level had reached the desired value. The dogs produced from 15 to 30 gm Hb per week and remained in good condition despite the severe anemia. The reticulocyte percentage was increased while the saturation index decreased. Percentage O<sub>2</sub> saturation of marrow blood, although variable showed no significant difference between the control and anemic periods. Similar results were obtained with arterial samples whereas jugular venous blood was less saturated, resulting in an increased A-V O<sub>2</sub> difference. From

these findings it appears that vigorous erythropoiesis is not necessarily associated with a lowered O<sub>2</sub> saturation of the blood circulating through bone marrow.

**The relation of initial blood pressure to adrenalin action.** D. M. GREEN, A. D. JOHNSON, A. LOBB and G. CUSICK (introduced by R. Frederick Becker) *School of Medicine, University of Washington.* Fifty-one patients whose systolic blood pressures ranged from 90 to 246 mm Hg were given a total of 80 infusions of adrenalin for period of 75 minutes' duration. Adrenalin was administered initially at an average rate of 0.2 micrograms per kilogram per minute. When the full effect had been obtained the rate was increased in steps of 0.2 mcg/kg/min until the individual limit of tolerance was reached. The infusion was then maintained at this rate until the end of the period. Blood pressure and pulse rate were measured every 3 to 5 minutes during infusion and at increasing intervals for 12 hours afterward. Response to adrenalin was calculated in terms of net and unit changes in blood pressure, pulse rate and systolic diastolic ratio. Individual variation was marked. However, when the patients were grouped according to initial pressure, no statistically significant mean differences in response were demonstrated. Post-infusion depression of blood pressure below the initial level was found to correlate more closely with the height of the initial pressure than with maximum pressure, maximum rise in pressure or maximum dose.

**Vasodilation produced by etamon, priscol, body warming and spinal anesthesia in normal extremities.** HAROLD D. GREEN and BEN C. OGLE (by invitation) *From the Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina.* Temperature of the exposed skin of the toes, feet, shin, knees, thighs, fingers, forehead and room temperature were recorded with an 8 point Leeds and Northrup micro-max over periods of 2 to 7 hours on patients in a constant temperature room ( $\pm 1-2^\circ\text{C}$ ) at environmental temperatures of 19-28° (av 24°C). Temperatures usually stabilized in 0.5 to 1.5 hours, at which time tetraethyl ammonium chloride (Etamon), 2 Benzyl 4,5 Imidazoline (Priscol), body warming or spinal anesthesia was begun. Body warming was by blanket and two 150 watt "reflector spot" lamps which strongly warmed the torso, but not the portions of the skin in which temperature was being measured. The most marked vasodilation was after spinal anesthesia. Etamon usually gave good vasodilation in doses of 500 to 1000 mg given I.V. over 20 to 60 minutes. 10 mg of Priscol was relatively ineffective, but 20 to 40 mg often produced striking vasodilation. Body warming produced further vasodilation after etamon.

### Studies of galactose and glucose metabolism

LAWRENCE GREENMAN and JOHN C RATHBUN (introduced by T S Danowski) *From the Department of Research Medicine, University of Pittsburgh School of Medicine, the Renzhausen Foundation, and the Children's Hospital of Pittsburgh* Galactose and glucose metabolism has been studied in a four month old white male infant with inability to handle galactose normally. The patient had cataracts, anemia, malnutrition, hepatomegaly, and ascites. In each of the studies a 50 per cent solution of galactose in water, 12 grams per kilo body weight, was injected intravenously during a 3 minute period. 15, 45, and 75 minute samples of venous blood were analyzed for galactose and total sugar. Glucose was then calculated from the two values. Prior to galactose restriction the 75 minute level of galactose in venous blood was found to be distinctly elevated (176 and 156 mgm per cent). Glucose alone, insulin alone, and glucose and insulin together were then administered before the galactose and in each instance increased the rate of removal of galactose. The 75 minute levels were decreased significantly towards normal, but were still definitely above the values observed in control subjects. Alcohol did not affect the galactose tolerance. Standard glucose tolerance tests were unremarkable. However, in the galactose studies with insulin and with alcohol described above, glucose decreased to hypoglycemic levels. Galactose restriction over a four month period did not alter the galactose tolerance, but the child gained in height and weight, the liver decreased in size, the anemia and ascites disappeared, and the cataracts regressed markedly.

**Effects of brain anemia on cortical oxygen consumption in vivo** R G GRENELL, P W DAVIES (by invitation), and D W BRONK *Johnson Foundation, University of Pennsylvania* Varying periods of anemia from fifteen seconds up to relatively long intervals, were induced in cats under dial anesthesia, by temporary compression of one common carotid artery following ligation of the second common carotid and both vertebral arteries. Readings of the rate of oxygen consumption before and after the anemia were made with the oxygen electrode which was placed over arterioles on the suprasylvian gyrus. Fifteen seconds of anemia induce no significant change in the oxygen consumption. Thirty seconds of occlusion result in an immediate but reversible depression of the oxygen consumption down to a factor of one half. The depression is followed by a period of steady recovery, both phases extending over a period of approximately twenty to twenty-five minutes. An anemia of one minute duration is followed not only by a much sharper drop in oxygen consumption, but also by a recovery period which lasts over a long

period of time. More severe anemias produce irreversible depression.

Histological evidence has shown that permanent damage occurs in the cerebral cortex in as little as two minutes of complete anemia. The present findings indicate that severe depression of the oxygen consumption may result from one minute of anemia. Thus, in addition to observations of electrical activity changes and histopathology consequent to anemia of the brain, direct measurements of the oxygen consumption in vivo offer further evidence of the marked sensitivity of these cells to anoxia or arrest of the circulation.

**Electrical correlates of psychiatric disturbances** R G GRENELL, B MOORE (by invitation), H S BURR (by invitation), W BROWN (by invitation) and S FRIEDMAN (by invitation) *Divisions of Neuroanatomy and Psychiatry, Yale University School of Medicine and Fairfield State Hospital, Newtown, Conn.* In recent years it has been demonstrated that many physiological and pathological events produce measurable changes in the electrical properties of the organism. In many such instances, particularly in cases of psychiatric aberrations of the central nervous system, AC measuring techniques have yielded disappointingly little information of value. As a consequence of this, and in view of the interesting shifts in DC potential seen accompanying nerve injury and regeneration, it was deemed advisable to study DC potential differences in psychiatric patients under various conditions. With the Burr-Lane-Nims DC micro-voltmeter and reversible non-polarizable silver-silver chloride electrodes, a standard series of 8 measurements between areas on the head and chest was made in 61 controls (non-hospitalized, random individuals) and 150 psychiatric cases, over 100 of which were diagnosed as schizophrenics. In a relatively small group of patients, readings were made throughout a course of electric shock treatments; in others, readings were made before, during and after insulin shock. Two criteria were used in analyzing the data: a) arithmetic sum—the sum of the 8 potential differences in millivolts without regard to sign, and b) range—the difference between highest positive and lowest negative reading in each set of measurements.

Although the investigation is in its most elementary stages, and many variables have therefore not been taken into account (age, sex, etc.) the data thus far obtained appear to be of enough significance to justify further detailed studies of both clinical and fundamental physiological nature. There appears to be a definite correlation between the measured potentials and the psychiatric status of the individual (control sum mean = 11, schizophrenic sum mean = 65; control range is + to - 5, patient range as far out as + 30 to - 30).

In other words, the method demonstrates electrical correlates of psychiatric conditions which are of quantitative nature. In successfully treated cases, electric and insulin shock have shifted the abnormal potential levels of the patients back to those of normals. The fact that insulin pushes abnormally high potentials down into the normal range, gives suggestive evidence of an important physiological nature, namely, the relationship between potential levels and metabolism.

A simple, relatively rapid test appears to be at hand for use in the clinic as an aid to psychiatric diagnosis and evaluation of therapeutic procedures. It is also felt that the observations present an important clue to the possible physiological nature of certain psychiatric conditions, as well as suggesting an extremely provocative relationship between DC potential and metabolic levels.

**Hepatic lymph and ascitic fluid following experimental chronic obstruction of the inferior vena cava.** J. H. GRINDLAY, E. V. FLOCK and J. L. BOLLMAN. *Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.* In other studies of hepatic lymph we observed greatly increased flow in dogs with carbon tetrachloride cirrhosis of the liver accompanied by ascites. Our experiments suggested the possibility that ascitic fluid is the result of venous congestion in the liver and that this fluid comes largely from the liver or the extrahepatic liver lymphatics. Accordingly, in order to produce a slowly progressive venous congestion in the liver, we wrapped cellophane bands loosely about the inferior vena cava above the diaphragm in a series of dogs. Six to eight weeks later these dogs had ascites, greatly enlarged and dilated liver lymphatics, and large, granular livers. Although the liver in each case showed venous engorgement, the spleen, portal veins and mesenteric lymphatics were not engorged. The rate of flow of hepatic lymph was greatly increased in these dogs and each had two to three liters of free fluid in the peritoneal cavity. Both lymph and ascitic fluid were quite clear, the former faint yellow, the latter faint pink. Plasma protein and cholesterol concentration in most dogs were slightly low. Plasma phospholipid was normal and alkaline phosphatase, although there was obvious liver damage, was not elevated. Protein, cholesterol, phospholipid, and alkaline phosphatase concentrations were lower in hepatic lymph than in plasma and lower in ascitic fluid than in lymph.

**Relationship of volumetric rate of blood flow to arterial diameter.** RICHARD A. GROOT. *Department of Anatomy, The Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina.* It was desired to find if, (1),  $Q = kD^n$ .  $Q$  is flow-rate to which the prevailing inside diameter,  $D$ , of an artery is adapted,  $k$  and

$n$  are constants. The validity can be tested by measuring arteries participating in dichotomizations, calculating for each branching the equating value of  $n$ , ( $n_{eq}$ ), in  $D_a^n = D_b^n + D_c^n + \dots + D_e^n$  where  $a$  signifies parent vessel, the other subscripts, daughter vessels. If  $n_{eq}$  be found constant, then  $n$  in  $Q_a = kD_a^n$ ,  $Q_b = kD_b^n$ , etc., will be the same for all dichotomizations. Each daughter is in turn a parent,  $k$  will be the same for all dichotomizations. Some variation of  $n_{eq}$  will not negate suitability of (1), since biological processes involved in adaptation of  $D$  to  $Q$  might be expected to lack precision. Furthermore, variation in  $n_{eq}$  may be much greater than corresponding variation in  $n$  in (1). Vessels from 24 to 3200 microns were recorded, the pulmonary circulation and aortic arch branchings were excluded. The mean of  $n_{eq}$  was 2.6, which established the mean of  $n$  as 2.6. Extremes of  $n_{eq}$  were 2.1 and 3.1. Greatest discrepancy of  $D$ 's at a given dichotomization from the calculated diameters necessary for  $n_{eq}$  to equal 2.6, was 3.18%. Calculated limits of  $n$ , with  $D$  in microns, were  $2.6 \pm 0.02$ . Thus,  $Q = kD^n$ . Involving Poiseuille's law, we obtain  $(P_1 - P_2)/L = k u / D^4$ , which, even with due consideration to decrease in viscosity,  $u$ , in tiny vessels, shows that pressure drop per unit length is always greater in a daughter than in its parent.

**Hepatic arterio-venous oxygen differences in patients with normal and diseased livers.** A. E. GROFF, (by invitation), E. DEF. BALDWIN, (by invitation) and S. E. BRADLEY, *From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital in the City of New York.* Simultaneous collection of hepatic venous blood (by venous catheter) and arterial blood were made during the determination of estimated hepatic blood flow (EHBF) by Bromsulfalein clearance in nine normal human subjects and in five patients with portal hypertension due to cirrhosis. Normally, the oxygen A-V difference average 3.4 ml per cent, ranging between 2.5 and 4.5 ml per cent. The splanchnic oxygen consumption averaged 50.9 ml per min and varied between 38.6 and 68.2 ml per min. When hepatic blood flow was reduced by disease (5 patients with portal hypertension), the average A-V difference was 5.3 ml per cent (3.4 - 7 ml per cent). This increment was probably not attributable to an increased proportion of portal venous blood in the hepatic venous outflow as a result of the elevated portal pressure, since in one patient with Banti's syndrome due to a splenic arteriovenous fistula who had a normal liver at necropsy, EHBF and the hepatic oxygen (A-V difference) were within normal limits. The portal venous contribution was eliminated in another patient by ligation of the portal vein at the time of porto caval anastomosis two years prior to study. In this instance, an EHBF



of 460 ml per min was associated with an oxygen A-V difference of 6.3 ml per cent, despite an exclusively arterial hepatic blood supply. Thus, it appears that the hepatic oxygen A-V difference in man is, in part at least, a function of the rate of hepatic blood flow.

**Inhibition of gastric secretion by "histaminase"** M. I. GROSSMAN, C. R. ROBERTSON (by invitation), and W. L. ANDERSON (by invitation) *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago*. Previous attempts to counteract histamine stimulated gastric secretion by injection of histaminase preparations have been unsuccessful. A new, more highly purified and less toxic histaminase preparation (Winthrop) was used in the present studies. This material, prepared from hog kidney, contained approximately 1 Winthrop unit of histaminase activity per milligram of dry powder. Intravenous injection of 50 units per kilogram into dogs with gastric pouches receiving 0.0125 mg. of histamine dihydrochloride subcutaneously every ten minutes resulted in 90 to 100 per cent inhibition of the gastric secretory response for from 1 to 6 hours. The gastric secretory response to food and to parasympathomimetic drugs was similarly inhibited by the histaminase preparation. Purification of the product resulted in a material free of emesis producing and vaso-depressor substances. Although the gastric secretory depressant is destroyed by boiling, it is not yet certain whether the depression of gastric secretion is due to the histaminase or to some other constituent of the extract.

**Preganglionic B fibers to the inferior mesenteric ganglion of the cat** HARRY GRUNDFEST and MINCO AMIEL (by invitation) *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York*. The hypogastric nerve of the cat contains both B and C fibers, whereas the preganglionic nerves to the inferior mesenteric ganglion have A and B components. The C fibers of the hypogastric nerve therefore are clearly postganglionic, but the nature of the B fibers in the nerve has been uncertain. Oscillographic techniques with graded antidromic stimulation of the hypogastric nerve in intact or excised ganglionic preparations shows that most, if not all the B fibers of the nerve are preganglionic. Their electrically evoked response ascends to the ganglion, and stimulates the latter, as is evidenced by a response descending the nerve in C fibers. The origins of the respective electrical activities are evidenced by the phase of the ascending and descending potentials. Preganglionic B fibers thus can make synaptic connections with cells in more than one autonomic ganglion by terminations *en passant*.

**Diet and gastro-intestinal symptoms at altitude** M. MASON GUEST *Wayne University, College of Medicine*. The effect of a bland diet upon the inci-

dence of gastro intestinal symptoms during a 3 hour sojourn at a simulated altitude of 38,000 feet was tested in a group of 2,448 healthy men between the ages of 18 and 30. The diet, consisting of fruit juice, cereal (bran excluded), white toast, jam or jelly, lean beef or fish, baked or boiled potato and milk or tea was fed at meal time during the 24 hour period preceding the decompression. The subjects, under supervision at all times, were not permitted to eat between meals or drink carbonated beverages or milk shakes. The control group was obtained by random sampling of the military population which furnished the experimental subjects. These individuals were not warned of the possible effect of diet upon gastro intestinal symptoms and were provided with army garrison rations with no restrictions on between meal feeding. The control sample consisted of 1,915 men.

| Symptoms                  | Control  | Experimental |
|---------------------------|----------|--------------|
|                           | per cent | per cent     |
| Total gas pains           | 6.6      | 6.7          |
| Severe gas pains*         | 1.9      | 2.4          |
| Total nausea and vomiting | 4.0      | 3.7          |
| Severe veno embolism*     | 11.7     | 13.3         |

\* Recompression because of severity of symptoms

Since the bland diet had no effect in promoting a significant reduction in the incidence of symptoms, a re evaluation of the effect of diet upon gastro-intestinal irritability may be warranted.

**The quantitative effect of hemorrhage on plasma proteins** C. C. GUTHRIE and MARIAN E. LEE (by invitation) *Dept. of Physiology and Pharmacology, School of Medicine, Univ. of Pittsburgh*. These results were obtained on blood samples removed from six etherized dogs during measurement of their blood volumes by a modified Weleker method. In three, the proteins were calculated from specific gravity of serum, but in the others they were also determined by heat coagulating, drying, extracting with chloroform and ether and with water. The results by the two methods did not significantly differ. About forty minutes after the first sample was drawn, a total quantity equivalent to about 40% of the original blood volumes was removed in 7 samples. At this time, the circulating and removed proteins showed a percentile increase over the original amount of from 7.9 to 16.6, averaging 11.48. Following this, bleeding was continued and 0.9% NaCl solution intravenously injected until death, which occurred about thirty minutes later, at which time the protein total increase was 28.4 to 39.4%, averaging 34.6. In three experiments, fibrin was determined by whipping the blood samples, washing, drying and weighing. After hemorrhage only, there was an increase of

14.6 to 43.7%, averaging 29.1. At death, the total increase was 41.6 to 70.5%, averaging 60.2.

**Resistance characteristics of rectifier element in single nerve fibers** RITA GUTTMAN *Brooklyn College, Brooklyn, N. Y.* In 1941 it was shown that the nerve fiber membrane acts as an electrical rectifier, permitting current to flow more easily outward than inward. The rectifying property was found to decrease reversibly on narcotization or treatment with K and to disappear on death, when the membrane acts as an ohmic resistance. The present study investigates the resistance characteristics of the rectification curve in an attempt to discover whether the anodal or cathodal portion or both are affected on loss of rectification.

One end of a single nerve fiber of the squid was dipped into isosmotic KCl, killing that end and removing its rectifying properties. The other end was placed in sea water or experimental solution. The interelectrode stretch was in oil. Resistance offered to an E.M.F. of varying magnitude sent into the fiber first in one direction and then in the other was measured by means of a direct current Wheatstone bridge and calculated currents were plotted against applied voltages. Anodal currents and voltages were termed positive and cathodal negative. Plotting voltage against current, the experimental curve deviates more from the straight line which would represent an ohmic resistance in the first quadrant, where current and voltage are positive, than in the third quadrant, where current and voltage are negative. On narcotization, treatment with K or death, the anodal portion of the curve changes more than the cathodal as a straight line is approached. As time elapses, excitability is lost first. Then the resting potential and rectification concurrently disappear.

**Glutamic acid in neural activity** C. HABER, L. SAIDEL, (introduced by R. W. Gerard,) *Dept. of Physiology, The University of Chicago*. A microbiological method has been applied to the determination of free and combined non-protein glutamate in nervous tissue. The tissue is homogenized and proteins precipitated with a minimum of tungstic acid. Samples of the supernatant are assayed, with and without prior acid hydrolysis, by addition to a modified Snell and Wright medium (*J. Biol. Chem.*, 1944, 151: 193, 1941, 139: 675) adequate for growth of *Lactobacillus* 17.5, except in its glutamate content. These mixtures are inoculated with the bacteria and the 72 hr. turbidities, read with a photoelectric colorimeter, compared to those obtained with standards of glutamate-medium mixtures. Brains of rats killed by decapitation contain about 125 micrograms free glutamic acid and 220 micrograms total non-protein glutamic (free and combined) per 100 mgms. tissue, wet weight (standard error  $\pm 4$  micrograms). Preliminary results indicate that strychnine con-

vulsions produce approximately 30% decrease in free or combined glutamic acid in brain. Effects of other changes of neural function and metabolism on glutamate concentration in brain and nerve will be discussed.

**The phosphatide composition of human erythrocytes** M. H. HACK (introduced by Hubert R. Catchpole) *Dept. of Pathology, Univ. of Illinois, College of Medicine, Chicago*. It was recently shown (*J. Biol. Chem.* 169: 137, 1947) that the phosphatide composition of human erythrocytes could be determined indirectly. The data of the present report was obtained by direct assay, and confirms the results previously obtained. Erythrocytes were obtained by centrifugation of citrated blood and the cells washed repeatedly with physiological saline and dried by (1) lyophilization (2) acetone. The dried cells were extracted with chloroform-methanol and the extracts assayed for the component phosphatides by the method previously described. The data is expressed in mM/liter of cells calculated from the dry weight equivalent, which was found to be approximately 360 grams per liter of wet packed cells.

| Phosphatide   | Lyophilized |          | Acetone dried |
|---------------|-------------|----------|---------------|
|               | Direct      | Indirect |               |
| Total         | 3.82        | 3.91     | 1.07          |
| Cephalin      | 1.57        | 1.72     | 0.51          |
| Lecithin      | 1.15        | 1.13     | 0.19          |
| Sphingomyelin | 1.10        | 1.06     | 0.37          |

The agreement between the two methods, using lyophilized cells, is evident. Drying by acetone extraction decreases the phosphatide content considerably, particularly the lecithin fraction. It was found that lyophilized erythrocytes swell tremendously and form a tough, rubber-like mass when wetted with chloroform-methanol, this mass could, however, be broken up in a Waring Blendor to facilitate the preparation of the extract. Although acetone-dried cells did swell slightly, they did not agglutinate.

**The brightness threshold as a function of area and receptor number in various retinal regions** C. HAIG and E. M. HAIG (by invitation) *Dept. of Physiology and Biochemistry, New York Medical College, New York 29, N. Y.* Measurements of the minimal brightness ( $I_c$ ) required for stimulation of dark adapted rods and cones were made, over a range of stimulus areas ( $A$ ) of  $1/10^7$ , in retinal regions from the center to  $18^\circ$  in the temporal periphery. For rod measurements the eye was completely dark adapted. For cones the eye was first light adapted, and measurements were made during dark adaptation in the period when cones are completely dark adapted but rods are still "non-functional." Wavelengths longer than 555 m $\mu$  were

used to avoid differential absorption of the violet end of the spectrum by the yellow macular pigment. To compute rod and cone numbers, Østerberg's (1935) counts of a single retina were employed. The following quantities for one eye are approximate. For small areas,  $I_t A = \text{Constant}$  in all retinal regions. For rods the relation holds rigorously for areas of subtense  $< 0.5^\circ - 0.8^\circ$  (2800 rods), and for cones for areas of subtense  $< 0.2^\circ - 0.5^\circ$  (600 central and 80 peripheral cones). Within these limits, for a constant visual effect (given threshold value) in all peripheral regions, the number of receptors is constant. Thus, for a constant, small area,  $I_t$  is inversely proportional to the receptor population density, i.e., to the concentration of photosensitive material. For larger areas,  $I_t$  approaches a constant minimal value ( $I_{\min}$ ) such that  $(I_t - I_{\min}) A = \text{Constant}$ . The data indicate that the brightness limin is inversely proportional to the exposed receptor surface area, summation of subliminal nerve impulse frequencies probably occurring when more than one receptor is exposed.

**Industrial efficiency as affected by various kinds of foods consumed during the mid-morning and mid-afternoon rest periods.** JOHN HALDI and WINFREY WINN (by invitation) *Dept. of Physiology, Emory University, Emory University, Georgia*. This investigation was conducted in a large cotton bag factory. All the subjects were women. They were engaged continuously throughout the working hours of the day sewing bags on electrically driven sewing machines. Their compensation was determined by the number of bags sewed. An arrangement of this work has the following advantages: 1. it provides a monetary incentive for maximum production, 2. the hourly output can be accurately determined by counting the number of bags sewed. On different days of the week the refectation was as follows: (1) cake, banana, milk, (2) frankfurter, bun, potato chips, soft beverage, (3) chocolate bar, (4) soft beverage only, (5) nothing. Only rough approximations were made of the caloric intake, but it is quite obvious that there was a marked difference in the amount and kinds of food eaten on different days. The experiment extended over 14 consecutive working days. The last scheduled experiment with no refectation was cancelled because of the complaint registered by the subjects. Owing to the wide variation in the work output from one subject to another, observations were discarded unless the subject took part in all the experiments of the week.

From the data obtained in 52 individual observations after each type of refectation, it is concluded that work output was not affected by the kind or amount of food eaten during the mid-morning and mid-afternoon rest periods. Abstention from food during the rest periods on the two days in which

this experiment was conducted likewise had no effect on work output.

**Studies on the mechanism of "acid rebound" in gastric acidity.** E. HALE (by invitation) and M. I. GROSSMAN *Department of Clinical Science, University of Illinois, College of Medicine, Chicago*. One possible mechanism of the alleged "acid rebound" in gastric acidity following the administration of sodium bicarbonate is metabolic alkalosis. The effect of 200 cc. of 2.6% sodium bicarbonate, when given intravenously, was observed in 12 experiments on 5 total pouch dogs. Secretion was established and maintained by repeated injections of 0.025 milligrams of histamine dihydrochloride every 10 minutes. There was no significant change in the average gastric secretion during the two hours of observation following a two-hour control period and one hour of infusion. The infusion of 1.8% sodium chloride in 10 control experiments demonstrated a moderate depression, in most instances, as compared with the control level of secretion. Another effect of the oral ingestion of sodium bicarbonate is the liberation of carbon dioxide. The possibility that carbon dioxide gas could shorten the emptying time of the stomach or stimulate secretion by distention or by a local effect on the parietal cell was studied. Two experiments were performed on each of 10 normal, fasting subjects. Two hundred fifty cc. of carbonated or uncarbonated distilled water were given alternately during the first or second hour following a 30-minute basal period. Carbonated water had no consistent or significant effect upon emptying time at 15 or 25 minutes or on the secretion of free acid. It is concluded that if "acid rebound" does occur it is not due to the changes in systemic acid-base balance or gaseous distention.

**The influence of substances affecting body temperature on thermal polypnea.** V. E. HALL, R. GRANT (by invitation) and J. FIELD *Department of Physiology, Stanford University, California*. In a study designed to determine whether the metabolic activity of the central nervous system is a factor determining the level at which body temperature is regulated, the effect of certain substances affecting body temperature on (a) oxidative and glycolytic activity of cerebral cortex (see abstract by Field, Peiss and Hall), and (b) the respiratory response to heat (thermal polypnea) were determined. Typhoid-paratyphoid vaccine, in doses evoking a fever of  $1-2^\circ\text{C}$ , raises the rectal temperature level at which thermal polypnea appears by about  $2^\circ\text{C}$ . If the rabbit is panting before injection, panting stops within fifteen minutes, but can be restored by raising the body temperature. The respiratory response to the inhalation of 95%  $\text{O}_2$  5%  $\text{CO}_2$  is not depressed by vaccine, so that the effect is presumably not on the inspiratory center. Persistence of normal rhythmic

respiration in vagotomized rabbits shows that the pneumotaxic mechanism is not inactivated by the vaccine. Magnesium chloride (3 mM per kg intraperitoneally) evokes a polypnea in rabbits at an environmental temperature of 20°C or higher, which does not appear at 10°. At both temperatures, the rectal temperature is reduced by 1-2°C. These facts, together with the ear vasodilatation evoked, suggest that magnesium chloride reduces the body temperature level at which heat defense mechanisms are activated. Similar studies of the effects of antipyrin, dinitrophenol and other substances affecting the body temperature are under way.

**Changes in radial arterial blood pressure during surgical resection of coarctation of the aorta.** G A HALLENBECK, E H WOOD and O T CLAGETT (by invitation). *Section on Physiology and Division of Surgery, Mayo Clinic, Rochester, Minnesota.* A mobile recording oscillograph which, with its associated pick-up units, can be transported to the operating room and used there without interfering with surgical procedures, has been designed to permit recording of physiologic data on patients undergoing operation. In its present form the apparatus can record arterial and venous blood pressure, using hypodermic strain gauge manometers, respiratory pressure changes within the anesthesia mask, using a similar strain gauge manometer with an air system, arterial oxygen saturation, using Millikan's compensated circuit oximeter, the electrocardiogram and the pulse rate, using a recording cardi tachometer, and appropriate time signals. The relatively large size of the instrument provides considerable flexibility and other recordings can be added as desired. Records were obtained of radial blood pressure changes during four surgical resections of aortic strictures. In two cases in which the left subclavian artery was temporarily clamped, severe acute hypertension occurred. In two cases in which the aorta was clamped proximal to the coarctation but distal to the left subclavian artery, no significant alteration in radial blood pressure resulted. With gradual opening of the vascular anastomosis which re-establishes aortic blood flow after resection of the stricture, radial blood pressure fell smoothly for a period of thirty to ninety seconds. The decrease in radial systolic blood pressure at this time ranged between 30 and 90 mm. of mercury.

**Comparison of the Fick and Dye injection methods of measuring the cardiac output in man.** W F HAMILTON, R L RILEY (by invitation), A M ATTIAH (by invitation), ANDRÉ COURNAUD, D M FOWELL (by invitation), A HIMMELSTEIN (by invitation), R P NOBLE (by invitation), J W REMINGTON, D W RICHARDS JR (by invitation), N C WHEELER (by invitation), and A C WITHAM (by invitation). *Department of Physi-*

*ology, University of Georgia School of Medicine, Augusta, and the Chest Service, Bellevue Hospital, Columbia University, New York.* The cardiac output was measured in a series of 48 experiments using the direct Fick procedure by means of intravenous catheterization. This was followed immediately by a determination using the dye injection method. The work was carried out in two laboratories, 18 comparisons being made in the Georgia laboratory and 30 in the Columbia laboratory as a result of a cooperative project in which two of the Georgia group (J W R and W F H) worked with the Columbia group in their laboratory. There was a wide variety of experimental conditions including normal subjects at rest and during exercise, and patients with mild congestive failure and various pulmonary abnormalities. The oxygen consumption varied from 182 to 1660 cc per minute, and the cardiac output varied from 2.5 to 16.8 liters per minute. The distribution of the results about the line of identity was almost symmetrical, so that the average of all the determinations by one method was nearly the same as by the other. The average scatter, 14%, was no greater than would be expected when the known inaccuracies of both methods are considered. The data include oxygen consumption, circulation time, mean circulation time, blood volume, volume of blood in heart, lungs and great vessels, as well as the cardiac output.

**Possible influence of cosmic energy cycles on growth.** FREDERICK S HAMMETT. *The Lankenau Hospital Research Institute, Philadelphia.* Abbot (Science, 105, 632, 1947) reports a solar constant of radiation with periodicity of 6 6456 days invariable, but with terrestrial effects subject to phase displacement of 1 or 2 days. There is week-to-week rhythmicity in extent of expression of basic growth and life cycle activities (initiation, proliferation, differentiation, organization, maintenance, regression, catabolism) in the marine hydroid *Obelia* (457198 hydranths, 35093 gonangia) during a 24-hour time slice in culture. The similarity of the rhythmicity cycle to that of solar radiation suggests it may be the product thereof. There is no open evidence rhythmicity is greatly conditioned by endogenous factors, or exogenous which are not derivatives of the solar cycle. Some valid interactivity difference exist. Rhythmicity of differentiation is less than that of organization or proliferation. Differentiation is cell specialization, organization is cell segregation, proliferation is cell multiplication. Each is expression of chemical processes specific and peculiar thereto. The differentials suggest processes concerned in cell specialization are more resistant to cosmic energy cycles than are those of cell segregation or cell multiplication. This fits the probabilities. For cell specialization is primary to ontogenetic growth,

and the most basic activity should be least sensitive to interference from exogenous factors. These observations are evidence that proper evaluation of the effects of cosmic (or atomic) energy on growth depends on knowledge, not of the reaction of growth as a whole, but rather on knowledge of the differential reactions of the components of growth.

**Relationships between the cerebral and cerebellar cortices in the cat** JOHN L HAMPTON (introduced by C N Woolsey) *Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore 5, Md* The projection of functional areas of the cerebral cortex onto the cerebellar cortex has been studied in the lightly anesthetized cat by means of the evoked potential technique. Using sodium pentobarbital as the anesthetic agent, the stimulating electrodes were applied to those points on the cerebral cortex from which the largest responses were obtained by stimulation of the appropriate peripheral receptor. The cerebellar cortex was simultaneously explored and the evoked cortical potentials, as observed by oscillographic methods, were recorded. The results show that 1 Auditory area I projects to the entire tuber vermis 2 Auditory area II (Woolsey and Walzl) projects to the entire tuber vermis. The latency of the evoked potential in this instance is somewhat less than for the auditory area I system 3 Somatic area I (the post central homologue) projects to the contralateral half of the anterior lobe 4 Somatic area II (Adrian, Woolsey) (i.e. the ant ectosylvian gyrus) projects to the tuber vermis, the pyramus and to the contralateral paramedian lobule 5 The cortical autonomic center or the eyes on the medial surface of the frontal pole (Siebens and Woolsey) projects to the contralateral cerebellar hemisphere (both Crus I and II), to the contralateral lobus simplex and to the contralateral half of the culmen, with the maximal responses occurring in the medial folia of the contralateral cerebellar hemisphere.

The cerebral cortical areas studied show considerable overlap in their cerebellar representation.

**Physiological adaptations to anoxia in congenital heart disease with cyanosis** J C HANDELSMAN (by invitation) RICHARD J BING, L D VANDAM (by invitation) *From the Department of Surgery, The Johns Hopkins University and Hospital* The arterial oxygen saturation of individuals with congenital heart disease and cyanosis is comparable to that of normal persons at altitudes ranging from 5000 to 30,000 feet. In both groups adaptations are directed toward restoring normal tissue  $pO_2$  (Barcroft), by decreasing the gradient between the  $pO_2$  of inspired air and the mean capillary  $pO_2$ . At high altitudes the atmospheric-arterial  $pO_2$  gradient is reduced primarily by hyperventilation. The gradient from arterial  $pO_2$  to mean capil-

lary  $pO_2$  (tissue  $pO_2$ ) is reduced principally because of the shape of the hemoglobin dissociation curve and to a lesser degree by an increase in the oxygen carrying capacity of the blood and in the cardiac output (Houston and Riley). In tetralogy of Fallot the cause of anoxemia is the intracardiac shunt rather than reduced barometric pressure. Thus, reduction of the gradient between the  $pO_2$  of inspired air and alveolar air is useless, and the only effective measures toward restoring normal tissue  $pO_2$  consist in reducing the gradient from arterial to mean capillary  $pO_2$ . Again the shape of the hemoglobin dissociation curve is most important, the increased oxygen carrying capacity of the blood much less so. The anatomical lesion limits the effect of the systemic flow. In contrast to normal oxygen uptake observed in persons at high altitudes, a lower basal metabolic rate is seen in patients with tetralogy of Fallot. The serum  $CO_2$  and  $pCO_2$  are low, the blood pH normal. This compensated  $CO_2$  deficit contrasts with the respiratory alkalosis seen at high altitudes.

**The failure of intravenous glucose to inhibit food intake in dogs** M L HANSON (by invitation) and M I GROSSMAN *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago* Experiments were performed on four mature dogs, standardized as to weight and daily food intake. The diet consisted of constant proportions of a dry commercial dog food mixed with cooked meat juice and it was given ad libitum for a 45 minute period at the same time each day. In one series of experiments from 200 to 400 cc of a sterile 20% glucose-0.3% NaCl solution were injected intravenously in one hour, daily for seven days. In other experiments a 15% glucose-0.3% NaCl solution was administered over a four hour period for three or more days, volumes in these instances depended upon the capacity of dogs to metabolize glucose (17 gms/kg/min) and ranged from 140 to 235 cc per hour. As a control for each experiment, equal volumes of an 0.85% or 0.6% NaCl solution were injected for similar lengths of time on days either preceding or alternating with those for glucose injection. All injections were concluded twenty minutes before feeding the animals. We observed no increase in rectal temperature or signs of restlessness or nausea which would suggest reaction. Decrement of food intake following injections, when it occurred, was of the same magnitude whether saline or glucose had been administered. In no case did the decrement exceed 25% after glucose administration, which change was within the range of spontaneous fluctuation of control food intake. Furthermore this decrement decreased with successive days of injection, presumably due to adjustment of the animal to the experimental procedure.

**Experimental investigation of pain intensity in labor** JAMES D. HARDY and CARL T. JAVERT (by invitation) *From the Russell Sage Institute of Pathology, The New York Hospital, and the Departments of Physiology, Gynecology and Obstetrics, Cornell University Medical College, New York, N. Y.* Using the method of estimating the intensity of the pain of labor by comparison with a pain of known intensity, measurements were made on ten women during the first and second stages of labor. Four of these patients received no analgesic. During the early part of the first stage of labor the pain intensity increased from three to five dols. In the later part of the first stage pain intensity attained a moderately severe level of approximately seven to eight dols. In the second stage of labor ceiling pain was experienced (ten and one half dols) during the period of uterine contraction.

The effect of certain common analgesics upon the pain intensity in the first stage has been studied and it was observed that although no pain threshold raising effect was achieved by the use of moderate amounts of the analgesics a marked decrease in the intensity of labor pain was produced for short periods following administration of the agent. As the decrease in pain is accompanied by lengthening of the interval between uterine contractions and a shortening of the contraction time, it is assumed that the principal effect of the analgesic was that of reducing the intensity of the uterine activity. In all the patients studied there appears to be a close correlation between the pain intensity and length and frequency of uterine contractions.

**Observations on the structure and function of the glomerulus in premature and full term infants** KENNETH HARE, LUISE UNGEWITTER (by invitation), HENRY L. BARNETT (by invitation), and HELEN McNAMARA (by invitation) *New York Hospital and the Departments of Pediatrics and Anatomy, Cornell University Medical College, New York City.* The anatomical development of the renal glomerulus has been followed in histological preparations from postmortem material from 29 fetuses and full term infants. The visceral layer of the epithelium is columnar in newly differentiated glomeruli and gradually passes through a cuboidal to a squamous form. Special silver staining reveals this layer to be a continuous sheet of extremely thin cells which contain a reticulum of argyrophilic fibrils. This process of differentiation is coincidental with the transition from the columnar to the squamous type of epithelium. It begins in the medullary layer of glomeruli and progresses toward the cortex to reach completion within a few months after birth. Rates of glomerular filtration were measured by inulin clearances in newborn premature and full term infants and at intervals thereafter until 6 months of age. These rates, low at birth, increased with age. The formation of a fine

reticulated investment of the glomerular capillary may be causally related to the increase in glomerular filtration.

**Factors responsible for transmission of visible light by the fibrous tunic of the eye** WILLIAM M. HART and BRUCE F. CHANDLER (by invitation) *Depts. of Ophthalmology and Biochemistry, Temple University School of Medicine, Philadelphia.* In early embryologic development, the sclera of the eye is transparent. This fact must be accounted for in any attempt to explain the transparency of the cornea in the definitive eye. Studies on hydration properties of the cornea have shown no relation between the extent of swelling and scattering of light. However, there are differences in swelling behavior between cornea and sclera which can be attributed to the peculiar structural arrangement of the collagenous fibers in the two tissues. It is a well known observation that pressure on the intact eye produces a clouding of the cornea which disappears immediately as the pressure is released. This phenomenon is a true birefringence. The chief factor concerned in transparency of the cornea is the presence of this birefringent system which is due to the orderly arrangement of the corneal lamellae. The sclera became opaque in fetal development as a result of its closely felted structure which distorts the optic axes of its fibers. Drying causes corneal opacities to disappear. When such corneas are rehydrated the opacities do not reappear. This effect is due to rearrangement of the distorted corneal fibers. The translucency of sclera on dehydration has the same significance as the effect of drying on corneal opacity.

**Retinal action potentials of photoreceptor cells and the discharge of nerve impulses in their axones** H. K. HARTLINE *Johnson Research Foundation, University of Pennsylvania, Philadelphia.* Single ommatidia, containing 10-15 retinula cells with  $\frac{1}{2}$  mm of their attached axones, were isolated in the compound eye of *Limulus*. An oscillograph and D.C. amplifier were used to record the slow changes in electrical potential between one electrode placed on the piece of cornea to which the distal ends of the retinula cells were attached and another electrode on the proximal end of the ommatidium where the nerve strand emerges (retinal action potential). Simultaneously impulses in the nerve strand were recorded by another amplifier and oscillograph. Upon illumination the corneal electrode became more negative relative to the proximal electrode, at the same time impulses were discharged in the nerve strand. There was a rough parallelism between the rise and fall of the retinal action potential and the frequency of discharge of impulses, different intensities of stimulation and different conditions of adaptation had comparable effects on both responses. Closer analysis, however, showed that the

parallelisms were far from exact, in many preparations it was possible to elicit large changes in the impulse discharge with only very slight concomitant retinal action potentials. It is not yet possible to decide whether the local currents accompanying the retinal action potential may be considered the direct cause of the discharge of nerve impulses by the photoreceptor cells.

**The utilization of carbohydrates by drosophila melanogaster** C C HASSITT (introduced by L E Chadwick) *Medical Division, Army Chemical Corps, Army Chemical Center, Maryland* *Drosophila* can survive for varying periods on pure solutions of single substances including pentoses, hexoses, di- and trisaccharides and the simpler polysaccharides, as well as on certain carbohydrate intermediates and other substances such as glycerol.

**In vitro action of adrenal cortical extract upon lymphocytes** OSCAR HECHTLER and DAVID STONE (by invitation) *Worcester Foundation for Experimental Biology, Shrewsbury, and Dept of Physiology, Tufts Medical School* It is now well established that adrenal cortical hormones (ACH) increase the rate of lymphocyte dissolution *in vitro*, principally within lymphoid structures (White and Dougherty, *Ann N Y Acad Sci* 46: 559, 1946). In previous work we have shown that addition of adrenal cortical extract (ACE) to blood has no direct effect upon blood lymphocytes, when, however, ACE was added to blood perfused through an isolated rabbit spleen, indirect evidence for accelerated lymphocyte breakdown was obtained, (Hechter and Stone, *Endocrinology*, in press). This finding suggested that the reaction of ACH to duce lymphocyte breakdown requires certain actors present in lymphoid tissue. In the present study, results will be presented which tend to show that the addition of ACE to breis or homogenates of rabbit lymphoid tissue, which are incubated with blood, accelerates the rate of lymphocyte breakdown, as evaluated by lymphocyte counts.

**The effect of adrenocorticotropin on renal hemodynamics and uric acid clearance** LEON HELLMAN (by invitation), RAYMOND E WESTON, DORIS J W ESCHER (by invitation) and LOUIS LEITER (by invitation) *Medical Division, Montefiore Hospital, New York 67, N Y* Purified adrenocorticotropin (Armour) was given by intramuscular injection (20 mg every four hours for ten doses) to four patients. Glomerular filtration rate, renal plasma flow, urica, uric acid and creatinine clearances, and sodium and chloride excretions were determined before and after each series of injections. All patients had reduced GFR and RPF initially. In two patients, one with proved and the other with suspected hypopituitarism, there was a marked increase in GFR, RPF, and uric acid

clearance after ACTH. In a patient with lymphatic leukemia, there was a marked increase in uric acid clearance and a significant, moderate increase in GFR and RPF. No change in GFR, RPF or uric acid clearance occurred in a case of pernicious anemia in relapse. Testosterone given to the patient with proved hypopituitarism caused only slight changes in renal function. All patients showed decrease in circulating eosinophils and/or lymphocytes after receiving ACTH.

**Further studies on the identity of the sustained pressor principle** O M HFLMER, ROBERT E SHIPLEY and K G KOHLSTADT *Lilly Laboratory for Clinical Research, Indianapolis General Hospital* Intramuscular or intraperitoneal injection of various kidney extracts in several animal species causes the appearance in the sera of a substance or substances which are capable of destroying or "neutralizing" renin and the sustained pressor principle (Shipley et al, *Am J Physiol* 149: 708, 1947). In nephrectomized cats the intravenous injection of the antisera will lower the arterial pressure which has been previously elevated by the injection of plasma containing the sustained pressor material. It has not been determined whether or not the substance(s) in antisera which neutralizes renin is also responsible for the neutralization of the sustained pressor principle. The antirenin titers of the antisera have been determined by assaying the quantity of unneutralized renin. The assay is based upon the production of angiotonin.

**Chronic hyperventilation in normal human subjects** A HEMINGWAY, E B BROWN, G S CAMPBELL, F GOLLAN and J O ELAM *Department of Physiology, University of Minnesota* Normal young adult male subjects were hyperventilated in a Drinker respirator for periods of 8 to 24 hours at ventilation ratios of 2 to 3. The ventilation was set at a value just below the threshold for hyperventilation tetany. Analyses of the blood were made for CO<sub>2</sub> content and capacity, pH, acid-soluble and inorganic phosphorous, as well as in some experiments, sodium and chloride. Respiratory volumes and rates were measured before, during and after the experiment. Weakness, fatigue, headache and abdominal pains were caused by the long-continued hyperventilation. The usual respiratory alkalosis, denoted by lowered CO<sub>2</sub> content and elevated blood pH, was noted but the most striking feature was a reduction of the serum and urine inorganic phosphorous. These results have an obvious bearing on the management of respiratory patients.

**The effect of blood acid-base changes on convulsive seizures** CHARLES D HENDLEY (introduced by Horace W Davenport) *Departments of Physiology and Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah* Metabolic acidosis or alkalosis was produced in rats by

feeding ammonium chloride, sodium bicarbonate, or sodium acetate. Respiratory acidosis was produced by administration of 10 per cent carbon dioxide in oxygen. Blood obtained by heart puncture was analyzed for pH and total carbon dioxide, and  $p\text{CO}_2$  and extra fixed acid or base were calculated. Electroshock thresholds were determined, and the components of maximal seizure patterns were timed. Extreme metabolic acidosis (fixed acid =  $24 \pm 6$  mM/l, pH = 6.81-7.06) raised the threshold 13 per cent ( $P = 0.01$ ), but the same degree of acidosis (fixed acid =  $23 \pm 2$  mM/l) had no significant effect on seizure pattern. Mild metabolic acidosis (fixed acid =  $6 \pm 1$  mM/l, pH = 7.25-7.40), mild or severe metabolic alkalosis (fixed base =  $7 \pm 2$  mM/l, pH = 7.42 - 7.57 and fixed base =  $15 \pm 1$  mM/l, pH = 7.53 - 7.61) and moderate to severe respiratory acidosis ( $p\text{CO}_2 = 48 - 101$  mm Hg, pH = 7.01 - 7.34) had no significant effect on threshold or seizure pattern ( $P$  is greater than 0.25). Moderate to severe metabolic acidosis or alkalosis in rabbits produced no significant effect on the resting EEG, on the subconvulsive Metrazol EEG, or on transcortical potentials evoked by condenser discharges. It is concluded that acute changes in blood acid-base balance must be very severe in order to cause any changes in the measures of cortical excitability used in these studies.

**Tactile localization in the thalamus of cat and monkey.** ELWOOD HENNEMAN and VERNON MOUNTCASTLE (introduced by Philip Bard) *Dept. of Physiology, Johns Hopkins University, School of Medicine, Baltimore 5, Md.* The evoked potential method has been used to study the localization of tactile sensibility in the thalamus. The potential changes produced there by discrete tactile stimulation of the body surface were picked up by a monopolar electrode, carried by a stereotaxic apparatus. These potential changes were amplified and introduced into a loud speaker. By this method the area of skin whose stimulation produced potential changes at any one point within the thalamus was outlined. The point of maximal response within this area was determined oscillographically. The brains were sectioned serially, and the sections used to construct a three-dimensional model of the thalamus, within which the course of the needle tracts was determined. The responsive area in the thalamus of the cat is confined solely to pars externa and the pars arcuata of the ventro lateral nucleus. Within these nuclei the volume of thalamic tissue devoted to a particular cutaneous area varies directly with the peripheral innervation density. The contralateral body surface is represented in great detail, with face postero-medially, and tail antero-laterally. The axial portions of the body from nose to tail are represented in continuity across the superior portion of the respon-

sive area. There is, thus, no reversal of the cervical segments in the thalamus. The extremities are represented inferiorly. Ipsilateral face and mouth are represented medially to their contralateral locations. No other ipsilateral responses were noted.

The study has been extended to the *Macaca mulatta*.

**The effect of pressure breathing on circulating blood volume.** (1) **Fluid loss into the tissue spaces.** J. P. HENRY, H. JACOBS (by invitation), H. MEEHAM (by invitation) and A. KARSTENS (by invitation) *Dept. of Physiology, University of Southern California, Los Angeles, California, and Aero Medical Lab., Air Materiel Command, Wright Field, Dayton, Ohio.* A constant elevation of the intrathoracic pressure of 20 to 60 mm Hg results in a corresponding increase in mean venous pressure throughout the body. This in turn leads to a disturbance of the normal fluid equilibrium between blood and tissue fluid with significant hemoconcentration. Hematocrit and hemoglobin estimations were made on venous blood from eight subjects submitted to pressure breathing for 30 minutes at various pressures. In all cases there was hemoconcentration which increased in proportion to the pressure employed. At 30 mm Hg mean intrathoracic pressure the fluid loss in 30 minutes in eight experiments was  $4.0 \pm 0.5$  cc/100 cc blood and at 45 mm Hg in twenty experiments this loss increased to approximately  $10.3 \pm 0.5$  cc/100 cc blood. It is concluded that significant hemoconcentration is induced by breathing at pressures in the range of 30 to 50 mm Hg for periods of 15 to 30 minutes.

**The effect of caloric level of refeeding on the recovery of work capacity following semi-starvation.** AUSTIN HENSCHEL, HENRY LONGSTREET TAYLOR and ANGEL KEIS *Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.* The effect of caloric intake level on rate of recovery of work capacity following semi-starvation was determined on 32 young men who lost 24 per cent of body weight in 6 months on a semi-starvation diet. Work capacity was measured by the Harvard Fitness Test. The average pre-starvation control Harvard Fitness Test score was  $64.1 \pm 17.2$ , time of run  $242 \pm 63.0$  seconds and work accomplished  $4501 \pm 1173$  kg meters. Values at the end of semi starvation, as per cent of control, were 28.4, 20.9 and 15.8 for the score, time of run and work accomplished, respectively. For 12 weeks of restricted rehabilitation the 32 men were divided into 4 groups of 8 men, the daily caloric intake of the groups differed progressively by about 350 Cal with the weighted average caloric intake for this period being 2440, 2795, 3160, and 3495 for groups Z, L, G, and T, respectively. The fitness score at 6 weeks of rehabilitation, as per



cent of control score, was 31.2, 33.3, 48.6 and 49.6 for groups Z, L, G, and T, respectively, and 43.2, 50.9, 64.6 and 63.0 at 12 weeks. Substantially the same inter-group relationships held for time of run and total work accomplished. Recovery was significantly slower in groups Z and L than in the higher caloric groups but there was no significant difference between groups G and T. Supplementation of half the subjects in each group with extra vitamins had no beneficial effect on the rate of rehabilitation.

**The effect of hyperthermia on the flow of thoracic duct lymph** J. F. HERRICK and J. L. BOLLMAN. *Division of Experimental Medicine, The Mayo Foundation.* Thoracic duct lymph was collected continuously from a small polyvinyl tube as a cannula in the thoracic duct. On the second or third day after operation the rate of flow was constant over a period of several hours when the unanesthetized rat was maintained at normal body temperature. The rate of flow was from 1 to 2 cc. each hour in the rats used. The rectal temperature was continuously recorded by means of a thermistor. Hyperthermia was induced by placing the rat in the radiation field of a microwave generator, the magnetron tube oscillating at a frequency of 2450 megacycles per second. The body temperature of the rat began to rise immediately and could be maintained at any desired level by altering the output of the generator. In most experiments when the body temperature was elevated more than two degrees C. in 15 minutes the flow of lymph was reduced for 15-30 minutes subsequently and then returned to the control levels. The return toward control levels occurred whether the hyperthermia was maintained or the animal allowed to return to normal body temperature unless lethal temperatures were reached. Hyperthermia was also produced by increasing the environmental temperature and humidity. The flow of lymph was similar to that observed with microwave induced hyperthermia.

**Further studies on the correlation between skin volume pulses and blood flow** A. B. HERTZMAN and W. C. RANDALL. *Department of Physiology, St. Louis Univ. Medical School.* The correlation between the photoelectrically recorded skin pulse and the skin blood flow as demonstrated previously on the finger has been studied in other skin regions in the following experiments: (1) total basal cutaneous blood flow as calculated from thermal data were compared with the average skin pulse which in turn was calculated from a representative sampling of skin pulses from the various areas of the body; (2) Presumably maximal cutaneous blood flows in the forearm during histamine or mecholyl iontophoresis as calculated from the venous occlusion data were compared with the maximal ampli-

tude of the skin pulses in the dilated areas. The two groups of data suggest that the flow equivalents of the cutaneous volume pulses as derived from previous calibration data on the finger are too large to be transferable to other skin regions. The lower flow equivalents in the latter may be due to differences in the vascular architecture since they are obtained at high and low flows. Fair uniformity in basal, resting and maximal flows is observed in the skin of the legs, arms and trunk. This suggests uniform vasomotor tone and an approximately equivalent vascular bed in those areas. Higher relative and absolute values for flows in the hands, feet and face under similar conditions may be due to a combination of vasomotor and morphological factors.

**Effects of oral cinchona alkaloids on circulation of dogs with experimental hypertension** EDWIN P. HIGHT. *Department of Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina.* Oral doses of quinine sulfate and quinidine sulfate of the order of 15 mg./kg. were given to dogs with experimental neurogenic hypertension. The effect of these drugs on the mean arterial blood pressure was determined by arterial puncture and a mercury manometer. The effective renal plasma flow and glomerular filtration rate were measured by p-amino hippurate and creatinine clearances. These alkaloids cause a marked fall in arterial blood pressure with a renal vasodilatation. The accelerated pulse rate is little affected. Normal control dogs show minor changes in blood pressure but a renal hyperemia and an increase in pulse rate. The effect of a single dose lasts 2-3 hours, but 4 doses at four hour intervals will cause the action to persist overnight. It can be maintained subsequently by two or three doses a day. During a course of such treatment the dogs continue to eat and behave normally. When the drug is discontinued after several days of administration the circulatory effects disappear in about 36 hours. The effective plasma concentrations of the alkaloids fall in the range of 1 to 3 mg./liter. In this range there is rough correlation between concentration and effect. Similar experiments are being carried out on dogs with experimental renal hypertension.

**Is heat death due to a myotoxic factor?** WILLIAM A. HESTAND and FORST D. FULLER (by invitation). *Laboratory of Animal Physiology, Purdue University, Lafayette, Indiana and Dept. of Zoology, De Pauw University, Greencastle, Indiana.* It has been reported (Fed. Proc. 5(1):43, 1946) that rats killed by immersion of the feet in water of 45°C. produce some substance(s) which if extracted from the skeletal muscles and injected into recipient rats will cause death in the latter. Our results do not corroborate these findings and

are stated here. Aqueous extracts of skeletal muscles of mice and rats exposed to heat were compared to similar extracts of muscles of control animals not exposed to heat and found to be no more toxic. Dialysates of such aqueous extracts when concentrated and injected into recipient mice cause a progression of symptoms about as follows: (1) opisthotonus of lower cord, (2) locomotor ataxia, (3) respiratory distress (panting, gasping, hiccoughing), (4) asthenia, (5) head tremors, (6) marked gasping, (7) convulsions, (8) death. It appears that part of the effects are due to stimulation of the central neuraxis beginning at the lower cord and progressing anteriorly until the animal dies of medullary paralysis. Statistical evaluation shows that no real difference exists between muscle extracts of heat-killed and non-heat-killed animals. The toxic symptoms are not due to intravascular thrombosis but resemble peptone injection. No increase was found in toxicity of the muscle extract in animals subjected to or killed by barrel "shock", suffocation, anoxia, as well as heat. Thus animals killed by exposure to heat appear to die from some cause other than a chemical substance toxic to the organism.

**Di-isopropyl fluorophosphate (DFP)** site of injection and variation in response. H. E. HIMWICH, and A. M. FREEDMAN (by invitation). *Army Chemical Center, Medical Division, Edgewood, Maryland*. Adult male albino rabbits under light pentothal anesthesia were injected into the femoral artery, femoral vein, carotid artery and portal vein with 0.4-0.8 mg./kgm. DFP in saline at rate of 0.01 mg./sec. In general, the most resistant animals were those injected into the portal vein. Even with the very high dosage of 0.8 mg./kgm. nicotinic and muscarinic effects were transitory. These animals made a prompt and uneventful recovery. The most sensitive group were those rabbits injected into the femoral vein. They displayed all the nicotinic and muscarinic effects of DFP toxicity and suffered a higher mortality than those rabbits injected into the femoral artery. The latter group when exposed to the lower dosage displayed only fibrillations in the leg supplied by the injected artery. In the higher dose range, systemic signs although marked were in each case less severe than in rabbits injected into the femoral vein with the same dose of DFP. The mortality of those animals injected into the carotid artery was similar to the femoral vein group. In all instances a marked and persistent unilateral meiosis was observed on the injected side. Half of the rabbits injected with 0.4 mg./kgm. in one carotid tipped their heads with their noses away from the side injected and made circular movements pivoting about on their hind limbs in a direction away from the injected side.

The mechanism of these effects will be discussed.

**Estimation of subcutaneous pressure in animals explosively decompressed to pressures of 30 mm. Hg.** F. A. HITCHCOCK, FLOYD M. BEMAN (by invitation) and JOHN A. KEMPH, (by invitation). *The Laboratory of Aviation Physiology, The Ohio State University, Columbus*. It has been noted that following explosive decompression of dogs to levels of approximately 30 mm. Hg a marked subcutaneous swelling develops. It has been postulated that this is due to the vaporization of body fluids. During other experiments, it has been noted that following explosive decompression there is a marked rise in venous pressure. It is thought that this rise in pressure may be due to an increase in the subcutaneous pressure. Arterial pressure is maintained at from 50 to 70 mm. Hg even though arterial pulse curves indicate that cardiac output ceases following explosive decompression. In preliminary experiments an attempt has been made to measure the subcutaneous pressure which develops during this swelling. Such determinations have been made both with the mercury manometer and with the electric capacitance manometer of Lilley. Results indicate that subcutaneous pressure following explosive decompression rises to a point between 29 and 34 mm. Hg when the ambient pressure is about 30 mm. Hg. Such pressures would be sufficient to prevent the vaporization of body fluids and it is believed that they are at least in part due to technical difficulties, perhaps the introduction of air under the skin at the time the needle is inserted. Crude determinations of the compositions of the subcutaneous gas indicate that it is chiefly water vapor.

**Brain and muscle potassium in relation to stressful activities and adrenal cortical function.** HUNSON HOAGLAND and DAVID STONE (by invitation). *Worcester Foundation for Experimental Biology, Shrewsbury, Mass.* Studies of the concentration of brain and muscle potassium of 300 rats were made under a variety of experimental conditions. Small (0.5 mg. per day) doses of desoxycorticosterone or of pregnenolone did not affect brain potassium in normal rats, desoxycorticosterone, but not pregnenolone, reduced the concentration of muscle potassium by 6.2%. Uncompensated adrenalectomy increased the concentration of brain potassium by 24.0% and of muscle potassium by 37.5%. Pregnenolone (0.5 mg. per day) lessened the increase in concentration of brain potassium in adrenalectomized rats by 10.0% and of muscle potassium concentration by 12.0%. The stress of prolonged swimming reduced the concentration of brain potassium by 4.4% and that of muscle by 6.1%. These together with the foregoing results, were all statistically significant ( $P < 0.01$ ). There was a suggestion that doses of 0.5 mg. per day of

both pregnenolone and desoxycorticosterone tended to prevent decrease in concentration of brain potassium due to prolonged swimming although the results were not statistically significant ( $P$  approximately 0.1). Pregnenolone also tended to prevent the decrease of potassium concentration in swim-stressed muscle but the  $P$  value was not significant (0.1 to 0.2). Desoxycorticosterone significantly augmented the decrease of concentration of muscle potassium in swim-stressed rats. Pregnenolone has been previously found by Pincus and Hoagland to enhance the efficiency of prolonged fatiguing psychomotor performance in man. Our present findings indicate that this substance exerts a homeostatic influence in the regulation of brain and muscle potassium.

**Conduction velocity of skeleto-motor nerve fibers to partially paralyzed muscles in human poliomyelitis.** ROBERT HODES *From the Department of Physical Medicine, Graduate School of Medicine, and the Eldridge Reeves Johnson Foundation, University of Pennsylvania, Philadelphia*. Maximal muscle action potentials, evoked by percutaneous stimulation of motor nerves and recorded by surface electrodes, were amplified by a condenser coupled differential amplifier and photographed from a cathode-ray oscillograph. Conduction velocity of the most rapidly conducting nerve fibers supplying the intrinsic muscles of the hand and foot was determined by stimulating the nerve at two sites, measuring the latencies of the muscular responses evoked by each shock, and dividing the latency difference by the distance between the points of stimulation. Amplitudes of electromyograms from some 300 weakened and normal muscles, when correlated with clinical evaluations of muscle power from these same muscles, indicated that the degree of paralysis in poliomyelitis could be reliably estimated by comparing potentials from paralytic muscles with those from corresponding normal muscles. A linear correlation was found between the conduction velocity of the fastest nerve fiber in the residual innervation of a weakened muscle and the maximal electromyogram obtained from that muscle. This proportionality existed in 70 experiments covering a wide range of conduction velocities (the lowest being 29 meters per second, or 48% of normal) and potential amplitudes (the smallest being 0.2 mV, or 2% of normal).

The close correspondence between conduction rate and degree of paralysis is best explained by selective viral destruction of motoneurons having axons of large diameter. Histopathological and other data militate against indiscriminate depression of axonal excitability as the cause of the slower-than-normal conduction velocity in nerve fibers supplying paralytic muscles in poliomyelitis.

**Muscle action potentials in human poliomyelitis before and after treatment by Billig's method**

ROBERT HODES *From the Department of Physical Medicine, Graduate School of Medicine, and the Eldridge Reeves Johnson Foundation, University of Pennsylvania, Philadelphia*. Twenty-two patients in the convalescent stage of poliomyelitis who had shown no change in muscle power for at least one year were selected for electromyographic study. Maximal muscle action potentials, led from surface electrodes, amplified by a condenser-coupled differential amplifier, and photographed from a cathode ray oscillograph, were obtained by percutaneous motor nerve stimulation. Changes from control action potential amplitude were followed 0.9 to 17.8 months after treatment by the "closed manual neurotomy" method of Billig. Electrical activity was never observed after operation in muscles which had not yielded action potentials before neurotomy. In partially paralyzed muscles the average potential amplitude, up to 4 months post-operatively, was less than the control. Four to 8 months after treatment the potentials were on the average slightly greater than before operation. At intervals greater than 8 months, operated muscles produced potentials whose average amplitude was 22% greater than their respective control values. The increase in potential size after 8 months was statistically significant. An occasional muscle, examined 1-1½ years after neurotomy, showed a slightly smaller potential than the control. Even in those rare cases indications were that continued recovery would eventually produce muscle power equal to or exceeding the pre-operative level. The neuromuscular block observed in these patients before treatment (Hodes, R. *Am J Med Sci*, 213: 509, 1947; *Federation Proc*, 6: 130, 1947) was either abolished or markedly reduced after operation.

**The pH of gastric mucous secretion after equilibration in vitro with alveolar air.** FRANKLIN HOLLANDER and FRANCES U. LAUBER (by invitation) *Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York City*. It was previously found that mucous secretion, collected from dogs' Heidenhain pouches after topical stimulation with various agents, possesses pH's as high as 9.2. When these data were grouped according to stimulus, the group means increased with increasing potency of the stimulus, the potency being judged by viscosity of the secretion. These observations are characteristic of this secretion as it occurs inside the gastric cavity, but such "alkaline" material probably undergoes  $\text{CO}_2$ -loss between secretion by the cells and collection from the pouch. Therefore, specimens collected in response to emulsions of eugenol (5%) and mustard oil (1%) were equilibrated with human "alveolar" air, and the resulting pH's determined. Similar studies were made on blood plasma. Loss of  $\text{CO}_2$ , absorbed during equilibration, was minimized by layering

mineral oil over samples during pH measurement. The plasma specimens had initial pH's (following CO<sub>2</sub> escape) of 7.68-8.11, which dropped to 7.3-7.6 after equilibration. For eugenol-stimulated mucus, the pH's fell to 7.4-7.5, the initial values being 7.9-8.6. For mustard oil secretion, the values dropped from 7.5-7.9 to 7.3-7.4. These results indicate that the pH of gastric mucous secretion after equilibration with "alveolar" air is constant and approximately the same as that of blood plasma. This suggests that the pH of gastric mucus as it exists inside the cell and at the instant of ejection into the gastric lumen is approximately that of venous blood.

**Autotomy as a test for toxic factor A. L. HOKINS** (introduced by L. V. Heilbrunn) *Marine Biological Laboratory, Woods Hole, Mass.* When the leg of a crab is crushed, the animal typically responds by severing the injured appendage. This is the well known phenomenon of autotomy. It is logical to assume that one of the primary stimuli to autotomy is the production of an injury substance (toxic factor) by the injured muscles of the leg. Evidence for this point of view is given by experiments in which injury substances from frog muscles were injected into legs of the crabs *Libinia emarginata* and *Sesarma cinereum*. Injection of sea-water into such legs caused no autotomy. Nor did the injection of KCl cause autotomy in *Sesarma*. Also in *Libinia* preliminary results indicate that the legs do not drop off after KCl injection. On the other hand, injection of extracts of frog muscle caused as much as 50% autotomy of the injected legs in *Sesarma* and 30% in *Libinia*. The method of extraction makes it very improbable that the extracts contained any acetylcholine (which is known to cause autotomy). These experiments suggest that the autotomy of crab legs may be used as a means of testing for the presence of injury substances.

**Standardization of a method for the production of experimental atherosclerosis in the chicken.** L. HORLICK (by invitation) and L. N. KATZ *Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Illinois.* The effect of varying concentrations of dietary cholesterol on the degree of hypercholesterolemia, the time of development of atheromatous lesions, and their severity were determined. Cholesterol feed-

ing was instituted in four week old Leghorn cockerels. The results are given in the table herewith.

Feeding of cholesterol in the dosages employed produced a marked rise in the blood cholesterol in 6-12 times the normal levels within one week. There was a relationship between the amount of dietary cholesterol and the degree of the hypercholesterolemia. The critical dose of dietary cholesterol for cholesterolemia is between  $\frac{1}{2}$  and 1%. Doses above this do not produce any greater degree of hypercholesterolemia. The earliest atheromatous lesions occurred within two weeks after the commencement of cholesterol feeding. The frequency of occurrence and severity of the lesions varied directly with the amount of cholesterol in the diet and the duration of the feeding. In any individual group the severity of the lesions closely paralleled the degree and duration of the hypercholesterolemia.

**Orthostatic hypotension following hot or cold baths.** STEVEN M. HORVATH *From Department of Physical Medicine, Graduate School of Medicine, University of Pennsylvania.* Transitory non-fatal collapse following severe physical effort is a familiar phenomenon in competitive sport. Similar syncopal responses are also observed in individuals given hot baths for therapeutics. However, observations dealing with the nature of this postural hypotension are still few. Subjects were immersed for a period of 20 minutes in a bath of either 18° or 40°C. Heart rate, blood pressure, cardiac output and body temperature measurements were made in the supine and erect (70°) positions before and after the bath. Position was always changed passively by means of a tilting ballistocardiograph. Approximately one-half of the subjects showed evidences of orthostatic hypotension following the hot bath. No subject showed such tendencies following the cold. The alterations in cardiovascular responses to changes in environmental temperature and posture will be discussed.

**Direct experiments on the relation between the form of the ballistocardiogram and the shape of the systolic velocity curve in the aorta of man.** ORVILLE HORWITZ (by invitation), ROBERT L. MAJOCK (by invitation), and ISAAC STARR *From the Department of Therapeutic Research and the Department of Medicine, University of Pennsylvania.* As the first step of necropsy the aorta was cannulated through the aortic valve by a cannula of 2 cm diameter, bent into a right angle and leading to a piston syringe so altered that the diameter of its outlet equalled that of the cannula. The cadaver was laid on the ballistocardiogram with the catheter in place. A reservoir filled with saline, placed 1.3 meters above the subject, was connected to a cannula in the femoral artery to keep the aorta full and provide a more normal pressure. An optical record of the piston's position at each instant

| Dietary cholesterol | Per cent of animals in each group which developed atheroma at intervals indicated |           |               |
|---------------------|---|-----------|---------------|
|                     | At five weeks   | Ten weeks | Fifteen weeks |
| Control             | 0   | 0         | 0             |
| $\frac{1}{2}\%$     | 25  | 50        | 66            |
| 1%                  | 66  | 100       | 92            |
| 2%                  | 75  | 100       | 92            |
| 4%                  | 100   | 100       | 100           |

of time was recorded on the same film with the ballistocardiogram. A large variety of "systolic" ejection curves was secured by pushing or striking the piston in various manners. We have over 40 such experiments in 4 cadavers. When the ejection velocity curve was smooth and reached maximum velocity early in "systole" the ballistic form resembled that found in healthy persons during life, except that there was no II wave and I was smaller in relation to J than usual. When maximum velocity was attained late, or when the curve was not smooth, the ballistic form was highly abnormal. So the form of the ballistocardiogram was completely dependent on the shape of the aortic velocity curve and the results conformed in general to our expectations from theoretical considerations.

The knowledge gained will permit a more exact interpretation of abnormal ballistic forms in physiological terms.

**Heating of human muscle tissue by micro waves**  
S. M. HORVATH, R. N. MILLER (by invitation) and B. K. HURT (by invitation). *From the Department of Physical Medicine, Graduate School of Medicine, University of Pennsylvania.* The present modes of local heating are extremely inefficient in respect to their ability to penetrate deeply into tissues. It has been shown, however, that the employment of high frequency electrical energy can heat deeply and presumably quickly (although this latter point has defied experimental proof). While short wave diathermy (waves of several meters) is not readily adaptable to the heating of small areas, recent availability of generators producing wave lengths in the centimeter range which can be focused on small areas offers a tool which could provide such localization. The evaluation of Micro Wave energy sources as a means of providing deep and localized heating of tissues was made on eight subjects. These waves were directed at the outer lateral surface of the thigh for a period of fifteen minutes with the power loads varying in different experiments from 32 to 112 watts. Surface, subcutaneous and muscle (1 to 2 inches) temperatures were measured prior to and immediately following the application of the heat source. Observations were continued for periods of one to two hours. Due to the properties of the waves it was not feasible to measure temperatures with thermocouples during the exposure. In general the increase in temperature in the surface, subcutaneous and muscular tissues was roughly equivalent, a matter of 3° to 4°C. In a number of instances subcutaneous temperatures were appreciably higher than elsewhere in the area, and values as high as 44°C were recorded. All temperature changes were definitely localized in the sense that no increases were observed outside the area immediately under the directors employed to

project the high frequency waves. As far as is known at present, their maximum depth of penetration appears to be approximately two inches. Muscle temperatures of the opposite side of the thigh did not differ appreciably from control values. No changes in body (rectal) temperature were observed even when exposure periods were lengthened to thirty minutes.

**Statistical analysis of filtration rate and renal plasma flow in normal dog and man**  
C. RILEY HOUCK. *From the Departments of Physiology, New York University College of Medicine, New York and the University of Tennessee College of Medicine, Memphis, Tennessee.* The filtration rate (creatinine clearance) and the effective renal plasma flow (para aminohippuric acid clearance) in 75 normal, trained, fasted, conscious female dogs have been analyzed. Related to surface area, filtration rate has a mean of 84.4 cc/min/m<sup>2</sup>, range, 43-133, standard deviation, 19.1, coefficient of variation (100 × standard deviation/mean), 22.6. Plasma flow has a mean of 266 cc/min/m<sup>2</sup>, range, 139-430, standard deviation, 66, coefficient of variation, 24.8. The coefficient of correlation (r) between these functions is 0.79. Related to body weight, filtration rate has a mean of 4.29 cc/min/kg, range, 2.15-8.32, standard deviation, 1.01, coefficient of variation, 23.6. Plasma flow has a mean of 13.51 cc/min/kg, range, 8.05-22.43, standard deviation, 3.26, coefficient of variation, 24.1. The coefficient of correlation is 0.73. In a comparable analysis in 61 normal men (data from H. W. Smith, *Lectures on the Kidney*, University of Kansas, Lawrence, 1943), filtration rate has a mean of 75 cc/min/m<sup>2</sup>, range, 51.4-103.2, standard deviation, 12.7, coefficient of variation, 16.9. Plasma flow has a mean of 403 cc/min/m<sup>2</sup>, range, 198-546, standard deviation, 78.5, coefficient of variation, 19.5. The coefficient of correlation between these functions is 0.82. The dog has a greater filtration rate than man on a surface area basis, but a smaller effective renal plasma flow. Therefore, the filtration fraction (filtration/flow) in the dog (0.317) is higher than in man (0.186). The correlation between filtration and flow seems only slightly better in man than in the dog.

**Chemical studies of sex steroid balance in human subjects**  
F. D. HUMM (by invitation) and W. T. SALTER. *From the Laboratories of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut.* When the sex steroid hormones in the urine are evaluated for normal subjects, cancerous individuals and patients exhibiting various endocrinopathies, characteristic values are obtained by chemical methods. In the normal female, near the menstrual period the value for "estroid" excretion is frequently close to the normal male range, whereas

at the time of ovulation it rises to a sharp peak. In general, however, the range of "estroid" excretion as determined chemically in the urine of young females is definitely higher than the range for normal males. Similarly the range of 17-ketosteroid excretion is usually definitely below that for males. This discrepancy between male and female becomes accentuated if  $1000 \times$  the ratio of "estroid" to 17-ketosteroids is determined.

The values so obtained by chemical methods are at least as significant as biological assays. Chemical methods have the advantage of greater convenience and somewhat greater accuracy. Characteristic chemical values for "estroids" (micrograms per 24 hours) and 17-ketosteroids (milligrams per 24 hours) follow:

Normal woman (aged 25) at ovulation 59 and 7.8

Adrenal virilism, female, aged 42 44 and 42.9

Gynecomastia, male, aged 27 8 and 7.9

Hypogonadism, male, aged 19 4 and 6.1

Hypopituitarism, male, aged 47 0 and 1.9

Fibroadenoma of breast, female, aged 26 10 and 14.6

Breast carcinoma, female, aged 54 4 and 3.4

Further detailed data in characteristic cases are discussed. In particular, the effect of the administration of stilbestrol and testosterone are noted.

**Muscular atrophy as a state of "local hyperthyroidism"** ERNST G. HUFF (introduced by Ernst Fischer) *Baruch Center of Physical Medicine and Department of Physiology, Medical College of Virginia, Richmond*. Several biochemical data suggested that the state of a denervated muscle can be regarded as a "local hyperthyroidism." Two possibilities were regarded: 1) The production of thyroxine in the thyroid gland has a greater effect on the denervated than on the normal muscle. 2) The extrathyroidal production of thyroxine in the denervated muscle is different from that in the normal muscle. Experiments on rats. Thyroxine (subcut, 2 weeks) accelerates, while thiourea inhibits the atrophy. The differences were small, but particularly in the case of thyroxine highly significant. The effects cannot be explained merely by movement of water. The differences are also significant by calculating on the basis of the dried muscle. Arsenic and 2,4-Dinitrophenol accelerate the atrophy also. The degree of atrophy when completely carbohydrate-free or fat free diets were given was the same as when mixed diet was given. Complete protein-free diet retards the atrophy. The differences were significant, for both wet and dry muscle weight. This could be the result of the lack of "specific dynamic action" of protein. Dose response curves for thiourea. At all doses (2.5-40 mg/100 g) the thyroid glands of control rats were slightly heavier than those of rats with both the ischiadic

nerves cut. The diet of those rats was dog chow, which is rich in iodine. By giving a low iodine content diet (Gluten, Cornmeal, Corn Oil,  $\text{CaCO}_3$ ,  $\text{NaCl}$ ), the dose response curves show the opposite relations. At all doses, the thyroid glands of control rats were slightly lighter than those of rats with both ischiadic nerves cut.

**Effects of tris-( $\beta$  chloroethyl) amine on respiration, carbohydrate and protein synthesis, and cell division in *Chilomonas*** JOHN O. HUTCHENS, BETTY PODOLSKY (by invitation) and T. M. McMAHON (by invitation) *Toxicity Laboratory and Department of Physiology, University of Chicago*. *Chilomonas paramecium* grows rapidly in pure culture in solutions containing carbon as acetate and nitrogen as ammonia. Effects of tris-( $\beta$  chloroethyl) amine (HN-3) on rate of oxygen consumption, utilization of acetate, utilization of ammonia, synthesis of starch, synthesis of protein, and rate of cell division in this organism have been studied. When HN-3 in concentrations of  $10^{-6}$  to  $5 \times 10^{-4}$  M is added to cultures and the cells incubated in the presence of the HN-3 and/or its hydrolysis products the following effects are observed: (1) Oxygen consumption is not significantly depressed. (2) Acetate utilization and starch synthesis are not significantly depressed. (3) Ammonia utilization and protein synthesis are markedly inhibited. (4) Cell division is inhibited. The net effect is an increase in cell size, each cell containing a greater amount and proportion of starch and a smaller proportion of protein. At times varying from 5-15 hours, depending on HN-3 concentration, the rate of cell division returns toward normal and the cells tend toward normal size and content.

When  $10^{-3}$  M HN-3 is employed, as above, death of the cells occurs within one hour. Brief exposures to this concentration followed by washing of the cells permits isolation of a small proportion of survivors. Pure clones so isolated show persisting attenuated rates of growth, respiration, and synthesis of components for at least several months.

**Reflex activities and peristaltic function of the esophagus** KAO HWANG (introduced by A. C. Ivy) *Dept. of Clinical Science, University of Illinois College of Medicine, Chicago*. We have previously reported that the cervical portion of the esophagus in the dog receives from the vagus a separate motor nerve supply which has been designated as the pharyngo-esophageal nerve and was found to be responsible for all the motor activities of the cervical esophagus, peristaltic or of any other origin. When a gasping type of respiration developed in anesthetized dogs, the cervical portion or sometimes the entire length of the esophagus manifested spasmodic contractions synchronous with the inspiratory phase. The

cervical component of this contraction was abolished after bilateral section of the pharyngo-esophageal nerve. Stimulation of the central end of the vagus nerve is known to produce reflex contraction of mainly the cervical esophagus. This was found to be an ipsilateral reflex, being abolished by section of the pharyngo-esophageal nerve of the same side. Under morphine and procaine infiltration anesthesia the peristaltic function of the esophagus of the dog could be studied by introducing a balloon through the stoma of an esophagostomy located at the base of the neck. Secondary peristalsis was demonstrated throughout the entire length of the esophagus. A swallowing reflex was shown to arise from the cervical esophagus on distension. The pharyngo-esophageal nerve was found to be merely the efferent pathway for the peristaltic function of the cervical esophagus, both primary and secondary. Section of the recurrent laryngeal nerves close to the cricoid cartilage abolished in most dogs the swallowing reflex and also the secondary peristalsis of the cervical esophagus while the primary peristalsis was preserved.

**Changes in urinary glucose and nitrogen following adrenalectomy in the force-fed diabetic rat.** D. I. INGLE and M. C. PRILSTRUP (by invitation) *Research Laboratories, The Upjohn Company, Kalamazoo, Michigan*. Earlier studies of adrenalectomy in diabetic animals indicated that the amelioration of the diabetes was due principally to a decrease in gluconeogenesis from protein, as evidenced by the striking decrease in urinary nitrogen. Consider (1) the abnormally high food-intake of the diabetic animal is depressed to subnormal levels by adrenalectomy, (2) the abnormally high nitrogen loss of the diabetic animal falls to normal when the wasting of glucose is stopped. On these grounds it can be postulated that the change in nitrogen balance following adrenalectomy is secondary to an improvement in the utilization of carbohydrate and to changes in food intake.

Partially depancreatized male rats of the Sprague-Dawley strain were force-fed a medium carbohydrate diet and given saline to drink throughout the experiment. They excreted an average of 3.5 to 4.0 grams of glucose per day. The nitrogen loss was greater than normal. Following adrenalectomy there was a sharp rise in nitrogen loss by the third day which coincided with a fall in urinary glucose. The urinary glucose disappeared and the urinary nitrogen fell gradually to normal values, but not below. A decrease in gluconeogenesis from protein, as evidenced by the change in urinary nitrogen, could account but for little glucose. Adrenalectomy either caused a change in gluconeogenesis from fat or the animals regained the ability to utilize (oxidation, storage or conversion) the carbohydrate of

the diet which they wasted into the urine prior to adrenalectomy.

**Further observations on the effect of pentobarbital and of an adrenolytic agent upon the survival of animals subjected to a procedure resulting in experimental hemorrhagic shock.** R. C. INGRAHAM and FRANKLIN ROHMIG (by invitation), and H. GOLDBERG (by invitation) *Department of Physiology, University of Illinois College of Medicine, Chicago*. We have previously observed that animals hemorrhaged to establish a blood pressure of 35-38 mm Hg and maintained at this constant level for 90 min, pass into irreversible hemorrhagic shock in 70% of all cases and die despite transfusion. It was further observed that carefully designed therapy with pentobarbital and dibenamine had a marked beneficial effect on the survival of these animals. It was desired in these experiments to attempt to evaluate the efficiency of these agents upon the survival of animals in which the impending shock state is established by hemorrhaging the animal until the residual blood volume had been reduced below the generally accepted 60% of normal. In this type of procedure no attempt is made to maintain the blood pressure at any given constant level during the course of hypotension. Twenty dogs were subjected to the Wolcott method of producing the hemorrhagic shock state. It was intended that three hours after hemorrhage, the animals would be reinfused with all the remaining withdrawn blood. It was found 55% could not withstand a period of hypotension of this length. Fatality rate for the entire series was 80%. In the second series of 20 dogs, 7 mg/kg of pentobarbital was injected slowly 10 min after hemorrhage. Additional smaller amounts were injected as required to maintain the animals in a light state of sedation. 40% of the animals in this series were not able to withstand the three hour period of hypotension. The fatality rate for this series was 70%. In the third series, the adrenolytic compound was administered in doses of 3 to 1 mg/kg 10 min after hemorrhage. 44% of the animals in this series either died or showed definite signs of dying from cardio-respiratory before the three hour period had elapsed. 56% withstood the three hour period of drastic hypotension and the fatality rate for the entire series was 73%.

**The effect of pressure breathing on circulating blood volume.** (2) **Distension of the venous reservoirs.** H. JACOBS (by invitation), A. KARSTENS (by invitation) and J. P. HENRY *Aero Medical Lab., Air Materiel Command, Wright Field, Dayton, Ohio*. In addition to inducing a loss of fluid from the circulation, an increase in venous pressure induced by pressure breathing distends the veins that lie outside the raised pressure area of the head and trunk thus pooling a certain por-

tion of the available circulating blood. The method of Ebert and Stead, J C L 19 561, 1940, was applied to an estimation of the volume of blood sequestered from the circulation during pressure breathing. An approximate determination was made by this method of differences of the volume of blood in the two legs and one arm, isolated by application of arterial occlusion cuffs. The results of comparison made between the volumes thus obtained without and with pressure breathing at 40 mm Hg for 2 minutes were variable. However, the approximate mean volume change due to blood sequestered in the legs and one arm in four subjects, each submitted to three series of estimations, was calculated at approximately 300 cc or 6% of the total blood volume. This change is in fair agreement with estimations made by plethysmographic and teeter board methods. When combined with the loss as determined from hemocentrification studies, the estimated total loss of circulating blood volume when breathing for 30 minutes at 50 mm Hg pressure amounts to as much as 15 to 20 per cent. Still higher pressures will lead to even greater losses of blood volume. These changes are in themselves adequate to account for the frequent development of syncope during periods of raised intrathoracic pressure.

The effect of parathormone on the renal tubular reabsorption of inorganic phosphate. IFTAKHAR JAHAN (Post graduate fellow of the Govt of India) (by invitation) and R F PITTS *Department of Physiology, Syracuse University College of Medicine, Syracuse, New York*. The effect of parathormone on the renal tubular reabsorption of inorganic phosphate has been studied in twelve experiments on two female dogs. Six experiments were performed with and six without parathormone, the latter serving as controls. In the ones with parathormone the extract (Lilly) was given subcutaneously in two injections of 3 cc (USP 300 units) each, separated by an interval of 6 hours, starting about 20 hours previous to the experiment. The plasma inorganic phosphate was varied over a range of 0.9 to 5.58 mM/liter by intravenous infusion of inorganic phosphate of pH 7.4. The rate of glomerular filtration was measured by creatinine clearance. Prior to the administration of phosphate there was no apparent difference either in the plasma level or in the rate of excretion of phosphate in the six control experiments and in the six in which parathormone was administered. In contrast, the plasma calcium level and the rate of calcium excretion were considerably elevated in the parathormone experiments in comparison with the control ones. When the plasma level of inorganic phosphate was increased from 0.9 to 5.58 mM/liter, the rate of renal tubular reabsorption of phosphate was the same in the parathormone

treated animals and in the controls within limits of experimental error. It is concluded that increases in plasma calcium level and in the rate of calcium excretion in parathormone treated animals are not related to effects of parathormone on the phosphate reabsorptive mechanism of the kidney.

Pulmonary gas exchange following ligation of a pulmonary artery in man. O H JANTON (by invitation), H P REDONDO (by invitation) and J C SCOTT *Department of Physiology, The Hahnemann Medical College, Philadelphia, Pennsylvania*. A 21 year old male at operation on 10/24/47 for ligation of a recanalized patent ductus arteriosus was found to have had a completely ligated left pulmonary artery (done in 1941) and a patent ductus. No gross pathology of the left lung was noted except for a residual adhesive pleuritis. The ductus was ligated and patient made an uneventful recovery. Angiography gave no evidence of left pulmonary circulation. On 11/18/47 samples of air were collected by a bronchspirometric catheter (Gebauer) simultaneously from the right and left sides at the end of a forced expiration following a normal expiration. Three pairs of samples were collected at intervals of 3 or 4 minutes. During procedure patient was uncomfortable and dyspneic. Average  $\text{CO}_2$  and  $\text{O}_2$  values for the left side were 3.74% and 19.14% respectively, for the right side 6.15% and 10.51%. Successive pairs of samples agreed approximately, although there was a progressive fall in oxygen on the right side. The difference in  $\text{CO}_2$  and  $\text{O}_2$  from room air, in samples obtained from the left side could have been due to mixing of the right and left lung air during periods of breathing between collection of samples as the two sides were sealed by inflatable cuffs only during the time of sampling. A bronchial artery anastomosis with pulmonary capillary bed may have accounted for  $\text{CO}_2$ ,  $\text{O}_2$  values on the left side but no evidence for this assumption is available. Normal tidal air was 879 cc which supports the assumption that little gaseous exchange was occurring on left side.

Thalamo-cortical systems and the electrical activity of the brain. HERBERT H JASPER and JAN DROOGLEEVER FORTUYN (by invitation) *From the Department of Neurology and Neurosurgery of McGill University and the Montreal Neurological Institute*. Local electrical activity from specific cortical areas, and from certain subcortical structures was recorded in cats and monkeys during light barbiturate anesthesia, or in unanesthetized animals following medullary section as described by Bremer. The electrical response of the cortex to visual, auditory, and somato-sensory afferent impulses was characterized not only by evoked potentials in local primary (and secondary) receiving areas, but also by a less well localized



rhythmic response in the receiving area as a whole, secondary responses in many other cortical areas, and by a desynchronization of rhythmic activity in associated areas. Under certain conditions it seemed that a large portion of the cortex reacted in some way to a single specific afferent stimulus. Local stimulation of thalamic relay nuclei produced similar local effects in their areas of cortical projection with less generalized response. Control of rhythmic activity over widespread areas of the cortex was possible by local stimulation of the intralaminar nuclei of the thalamus and the nucleus reticularis. The distribution of patterns of spontaneous electrical activity seemed to follow units of nuclear thalamo-cortical projection systems. Integration and synchronization of these units was affected by the intralaminar and reticular systems of the thalamus. The pattern of rhythmic electrical activity characteristic of a given cortical area was abolished if its thalamic nucleus was destroyed.

**Arterial pulses simulated in electrical analogues of the circulatory system** **KENNETH L. JOCHIM** *Dept. of Physiology, Univ. of Kansas, Lawrence*. Previous reports (Fed. Proc. 1:13, 1942, 5:52, 1946, 6:136, 1947) have dealt with arterial pressure pulses and flow pulses recorded from a simplified mechanical model of the circulatory system, and have presented a mathematical analysis of the behavior of such a model. It has been found possible to set up various types of pulsed electrical networks as analogues of the circulatory system. In these networks the circuit parameters of capacitance, resistance and inductance are analogous, respectively, to vascular distensibility, resistance to blood flow and mass of moving blood. Voltage and current pulses were recorded from these circuits and compared with corresponding pressure and flow pulses obtained on animals. Factors determining the wave form of the electrical pulses were analyzed and the possible influence of analogous factors on arterial pulses in the animal were indicated. The use of electrical circuit analogues in the study of the circulatory system has the following advantages: (1) with electrical networks it is relatively easy to simulate in the laboratory many of the complex characteristics of the circulation, (2) the various circuit parameters can be accurately controlled, and (3) precise measurements of all variables can be made.

**Studies on deceleration** **MILTON JOFFE** (by invitation) and **F. A. HITCHCOCK** *The Laboratory of Aviation Physiology, the Ohio State University, Columbus*. In order to investigate the physiological effects of rapid, severe deceleration it is first necessary to know the strength of the structural components affected and their resistance to deceleration forces. The literature on the subject is nil. Thoraces of normal human individuals

were obtained and the physical properties of the ribs investigated by means of the Olsen Universal Lever Type Testing machine which applied an anterior-posterior compression to the rib to the point of destruction. All ribs tested broke in the anterior third at an average value of about 15 lbs. Curves of load vs. distortion were drawn, and calculations of total energy absorbed were made. All ribs absorbed energy in the range of 10-100 inch-pounds. Obviously the thorax can absorb more energy, up to 1000 foot-lbs or more, and we conclude that the bony structures contribute little to the total strength. Bone ash determinations made on fat-free, dry bone showed no variation correlating with strength. The strength of the intact human thorax by means of simulated decelerations is being observed by means of the fluoroscope. Changes in the viscera are also observed and animal experiments to determine changes in cardiology (pressure and EKG) with similar constriction of the thorax and abdomen are under way.

**The thermal denaturation of tobacco mosaic virus in relation to hydrostatic pressure** **F. H. JOHNSON**, **M. B. BAYLOR** (by invitation) and **D. FRASER** (by invitation) *Department of Biology, Princeton University, Princeton, N. J.* The thermal denaturation of tobacco mosaic virus, as measured by decrease in soluble protein, at pH 7.0 in 0.1 M phosphate buffer, follows the kinetics of a first-order reaction at both normal and increased hydrostatic pressures. Pressure retards the reaction, the rate constant changing from  $0.00759 \text{ min}^{-1}$  at  $68.8^\circ\text{C}$  and normal pressure, to  $0.00136 \text{ min}^{-1}$  under 7,000 lbs per sq in, indicating a volume increase of activation,  $\Delta V^\ddagger$ , of very close to 100 cc per mole. Using this value for  $\Delta V^\ddagger$ , and 153,000 calories for  $\Delta H^\ddagger$  as reported by Lauffer and Price (*Jour. Biol. Chem.*, 133:1, 1940), rates calculated according to the Theory of Absolute Reaction Rates for various pressures up to 10,000 lbs per sq in, and  $72.5^\circ$  as well as  $68.8^\circ\text{C}$ , were found to agree closely with the data from experiments. The free energy of activation,  $\Delta F^\ddagger$ , at normal pressure was computed to be 26,200 calories at  $68.8^\circ$  and 24,819 calories at  $72.5^\circ\text{C}$ , with an entropy of activation,  $\Delta E^\ddagger$ , of 370.89 calories per degree. The  $\Delta V^\ddagger$  of 100 cc per mole is of the same order of magnitude as that which has been reported for proteins of much lower molecular weight than tobacco mosaic virus, e.g., human serum globulin as well as intracellular and extracted enzymes.

**Electrochemical determination of ionic diffusion through the synovial membrane** **N. R. JOSEPH** (by invitation), **C. I. REED** and **I. E. STECK** (by invitation) *Department of Physiology, College of Medicine and Department of Chemistry, College*

of Pharmacy, University of Illinois Electrochemical methods of determining diffusion potentials have been applied *in vivo* to the study of the synovial membrane. The electrodes were the Ag-AgCl type in identical 0.15 M NaCl solution. The reference electrode was connected to the same solution inserted subcutaneously through an insulated needle outside the knee joint capsule of dogs. The indicator electrode was connected by means of a saturated KCl agar bridge to solution within the joint. Electrical contact was made through an insulated needle inserted into the joint cavity. Solutions of various electrolytes of any concentration were introduced into the joint and the differences of potential observed. In addition, currents through external resistance were determined, with or without applied external E.M.F. Polarization effects were also observed. For most inorganic ions of the alkali, alkali earth and halogen series, the resting potentials are stable and reversible. The relative ionic mobilities correspond in many cases to those in water. Iodide gives a high positive potential indicating high mobility in the tissues, while calcium and magnesium ions were also quite positive, indicating low mobilities. Salicylate and benzoate showed lower mobility than chloride, the least diffusible of the halides. Their mobility relative to chloride was greater in tissues than in water. Calculations were based on the Planck-Henderson theory of diffusion potentials. Acetyl salicylate ion and its 5-brom derivative in dilute solutions made isotonic with glucose showed initially high positive potentials that rapidly drifted negative. Similar results were observed with CNS ion. Studies of currents through an external circuit give data on the stability of the diffusion potentials, on specific rates of transference of ions and on the resistance of the membrane.

**Determination of circulation rate in articular structures.** E. KAPLAN and N. R. JOSEPH (introduced by C. I. Reed). *Department of Physiology, College of Medicine and Department of Chemistry, College of Pharmacy, University of Illinois.* The experimental method is to increase the temperature within the knee joint of dogs and to determine the subsequent rate of cooling. Temperature is increased by electrically heating for one minute a needle inserted into the joint cavity. The needle is wound with a number of turns of fine insulated copper wire which conducts the heating current. This is regulated to raise the temperature of the joint about 5°C. After the heating period the temperature is allowed to equilibrate. Temperatures are observed by means of a thermocouple inserted through the needle. When the logarithm of temperature displacement is plotted against time of cooling a linear relation is found  $\log \Delta T = \log \Delta T^0 - kt$  where  $\Delta T$  is the

temperature displacement from equilibrium at time  $t$ ,  $\Delta T^0$  is the initial displacement when  $t$  is zero, and  $k$  is a constant. Theoretically  $k$  is proportional to the circulation rate and inversely proportional to the volume of the static fluid which is cooled. The relation is of the form  $2.303 k = (a + r/v)$ , where  $r$  is the circulation rate in cc per unit time,  $v$  is the volume of the static fluid in cc, and  $a$  is a constant proportional to the static cooling rate when  $r$  is zero. As an average for dogs,  $k$  is approximately 0.4 reciprocal minutes. Evaluation of the data shows that on the average  $a$  is 0.17 reciprocal minutes and  $r/v$  is 0.75. Thus the normal circulation rate in the articular structures is 0.75 cc per minute per cc of static fluid. Variations of rates under various experimental conditions such as external changes of temperature and administration of drugs have been observed.

**Seismography applied to the study of track running.** PETER V. KARPOVICH and NATHAN MILLMAN (by invitation). *Dept. of Physiology, Springfield College, Springfield, Mass.* A new method for the study of track running has been developed. It employs a number of seismometers placed along the track at 20 yard intervals and the direct measuring of the distances between the footprints left on lime sprinkled over the cinders. The impacts made by runners' feet on the track cause oscillations of the ground, which are picked up by the seismometers and are recorded on a portable electrocardiograph (Sanborn Cardiette). The time intervals are also recorded in  $\frac{1}{10}$  of a second. This method enables to calculate the following: a—velocity of running at each step, b—duration of each step, c—length of each step. A few preliminary tests indicated that in sprint running the step made with the right leg is usually greater in length and faster than that made with left leg. This is because most people are right handed and their left legs are stronger than their right legs. Left handed people will have the reverse. An exception was observed in one left-handed man who developed athletic habits of a right handed man and therefore had his left leg stronger than the right one. During distance runs no difference in the length of steps has been observed.

**A method of determining the spread of excitation in the ventricles of the dog's heart.** W. KAUFMAN (by invitation), H. M. CHERNOFF (by invitation) and L. H. NAHUM. *Lab. of Physiology, Yale University School of Medicine, New Haven, Conn.* In an lead, during the inscription of the QRS complex, an upward deflection indicates electrical effects preponderantly from a distal area, a downward deflection indicates electrical effects preponderantly from a proximal area, and no deflection indicates that excitation is occurring either in an intermediate area, or simultaneously in a proximal and distal region where the electrical effects are

equal, but oppositely directed. The ventricular region being depolarized at any given time can be identified by an analysis of the positions of instantaneous points of the QRS complex in three simultaneously recorded unipolar limb leads (Vr, V1, V6). The sequence of ventricular excitation can be determined by the analysis, at successive time intervals, of instantaneous points of the QRS complex in the three simultaneously recorded unipolar limb leads. In dogs with intact chests, the first region of ventricular excitation lies within the mid-portion of the left anterior ventricle, near the septum, then, excitation spreads successively to the right anterior ventricle, to the posterior septum and posterior right ventricle, and to the apex and left posterior ventricle. The regions last depolarized lie in the right anterior ventricle near the pulmonary conus, and in the uppermost portion of the left posterior ventricle. These findings obtained by an indirect method are in accord with the results of direct measurements of the order of ventricular excitation of the dog's heart.

The effects of insulin hypoglycemia and coma on human cerebral metabolism and blood flow. SYMOUR S. KETY, FRANCIS D. W. LUKENS, RACHIEL B. WOODFORD (by invitation), MEREL H. HARMEL (by invitation), F. A. FREYHAN (by invitation), and CARL F. SCHMIDT. *Departments of Pharmacology, Medicine and Psychiatry, University of Pennsylvania and the Delaware State Hospital.* Measurements of cerebral blood flow by means of the nitrous oxide technique permitted determination of cerebral oxygen and glucose utilization in 5 schizophrenics in the resting state and in hypoglycemia both with and without coma. Average results are presented in the table. The arterial glucose shows a progressive fall while the oxygen and carbon dioxide contents are not significantly changed. The mean arterial blood pressure and the cerebral blood flow were not significantly affected. There is a progressive fall in both oxygen

and blood glucose utilization by the brain as hypoglycemia and coma develop and these changes are statistically significant. The cerebral RQ is not significantly changed from unity throughout. The ratio of oxygen consumed to blood glucose utilized by the brain confirms the conclusion of earlier workers that glucose is the sole foodstuff of the resting brain. The increase in this ratio as hypoglycemia progresses indicates either the oxidation of some other substance or the utilization by the brain of its own carbohydrate stores.

**Tryptic activity of pancreatic juice after dilution with gastric juice.** ELIZABETH N. KING (by invitation), M. H. F. FREIDMAN, and I. J. PRINGS (by invitation). *Department of Physiology, Jefferson Medical College, Philadelphia.* In tests of human pancreatic function gastric and duodenal contents are collected separately. However, constant aspiration applied to the stomach does not always prevent dilution of the duodenal contents with acid gastric chyme. In normal subjects following secretin administration the duodenal contents show a high initial rise in trypsin concentration; this does not occur in patients with proved chronic pancreatitis. The question arose whether in normal subjects the tryptic activity of secretin-stimulated pancreatic juice could be reduced to the low values seen in chronic pancreatitis by admixture of duodenal contents with gastric contents. Tryptic activity and pH of dog's pancreatic juice and human duodenal contents were determined before and after serial dilution with 0.1 N HCl, gastric juice, gastric juice boiled to inactivate pepsin, or water. Reduction of tryptic activity by 20% occurred when pancreatic juice was diluted with water to 45% of its original concentration. Tryptic activity was reduced 20% or more only when the pH was depressed below 6.0, this required dilution of the pancreatic juice to 70% with gastric juice, boiled gastric juice, or acid. Following secretin injection to 70 normal fasting subjects, 92 to 210 cc duodenal contents and 1 to 38 cc gastric contents were recovered in one hour. It is concluded that probably only in patients with gastric hypersecretion would enough acid gastric juice be secreted to inactivate sufficient trypsin to give the low values for tryptic activity shown by the duodenal contents of patients with chronic pancreatitis.

**Effect of corticotropin on ovariectomized C3H mice bearing adrenal adenomas.** JOSEPH T. KING, CARMEN B. CASAS (by invitation) and CLAIRE J. CARR (by invitation). *Department of Physiology, University of Minnesota.* Female mice of the C3H strain castrated at an early age are known to develop adrenal cortical adenomas and to come into constant subestrus on full feeding. We have previously reported that, when restricted in caloric intake to 66% or less, the ovariectomized

|                                     | Control | Hypoglycemia without coma | Insulin coma |
|-------------------------------------|---------|---------------------------|--------------|
| <b>Arterial</b>                     |         |                           |              |
| Glucose mg %                        | 74      | 19                        | 8            |
| CO <sub>2</sub> vol %               | 52.1    | 50.6                      | 50.8         |
| O vol %                             | 17.4    | 17.9                      | 16.6         |
| Mean blood pressure mm Hg           | 94      | 86                        | 93           |
| <b>Cerebral</b>                     |         |                           |              |
| A VO vol %                          | 5.9     | 4.4                       | 2.8          |
| Blood flow cc/100 g/min             | 58      | 61                        | 63           |
| O consumption cc/100 g/min          | 3.4     | 2.6                       | 1.9          |
| Glucose consumption mg/100 g/min    | 4.4     | 2.3                       | 0.8          |
| R Q                                 | 0.95    | 1.10                      | 0.92         |
| O <sub>2</sub> /glucose consumption | 0.77    | 1.13                      | 2.38         |

mouse still develops the adrenal tumor seen in the full-fed animal but fails to show the evidences of estrogenic hormone secretion. We have also reported that the tumor bearing calorie restricted mouse does not respond to gonadotropin. The restriction involves only calories, the absolute amounts of protein, minerals and vitamins being normal.

In this study the mice, ovariectomized at 19-25 days of age and restricted in calories to 66%, were injected (at 13-14 months of age) subcutaneously with 0.1 mgm corticotropin (Armour, Lot No 32-D 1 mgm =  $0.71 \pm 0.09$  mgm La 1 A) daily for 19 days. Since no change had occurred in the vaginal smear they were then given 0.1 mgm twice daily for 9 days. The animals remained consistently in anestrus before, during and after the injections. Smears were made daily for three weeks after the last injection. Approximately one month later the animals were sacrificed and found to have adrenal tumors but immature uteri and vaginae. It is evident that in calorie restricted ovariectomized C3H mice the tumorous adrenal, which is presumably the source of estrogen in the full fed animal, is not responsive to corticotropin in the amounts used.

**Visceral capillary permeability changes produced in the dog by anaphylactic shock and venous stasis.** JOHN S. KIRK (introduced by K. E. Jochim) *Dept of Physiology, Univ of Kansas, Lawrence*. It has been shown by other investigators that an increased flow of thoracic duct lymph and liver engorgement similar to that observed in anaphylactic shock could be produced in the dog by clamping the hepatic veins. Comparison has been made between anaphylactic shock and conditions which cause hepatic engorgement with a fall in mean arterial blood pressure. These experiments were designed to make a further comparison of these conditions. Permeability alterations in the extrahepatic visceral capillaries drained by the portal vein (with the exception of the spleen), immediately following anaphylactic shock in the dog were compared with changes in the same area produced by the injection of histamine dihydrochloride and by mechanical occlusion of the hepatic veins and portal vein. Splenectomy was performed before each experiment. Determinations of hematocrit and plasma protein concentration (falling drop method) were made on arterial and portal venous blood. A loss of the larger molecules from the plasma was demonstrated in anaphylactic shock and following injection of histamine dihydrochloride within the first few minutes after the fall in mean arterial blood pressure. No loss of larger molecules from the plasma was demonstrable during the first few minutes after hepatic or portal venous stasis. From the hematocrits, the theoretical increase in

plasma protein concentration in the portal venous blood resulting from fluid loss alone was calculated and compared with actual changes, in order to indicate whether or not there was any loss of protein from the vascular bed.

**Differences in exocardial and endocardial electrograms of rabbit, dog and calf.** BRUNO KISCH, FRANZ M. GROEDEL (by invitation) and PAUL R. BORCHARDT (by invitation) *From the Biological Laboratory, Fordham University, New York*. Electrograms were taken under Nembutal narcosis simultaneously from the surface and the inside of the calf heart a few days after birth. These are compared with electrograms recently obtained with identical technique from rabbits and dogs by the same authors. They show similarities and some conspicuous dissimilarities. The ventricular complex obtained from the cavity of the left ventricle of the calf begins with a tall upstroke (R), that from the surface at the apex of the left ventricle with a deep downstroke, forming a QS. In the rabbit and dog it is exactly the opposite. The ventricular electrogram taken from the surface of the right auricle, from inside the carotid artery, aorta, right auricle and superior vena cava, in dog and rabbit, contains no or a small R, and a deep negative deflection (QR). In the calf, the corresponding electrograms and that obtained from inside the left auricle show no Q, a high R, while S may be present or absent. As in dogs and rabbits, it is possible to produce auricular fibrillation by the local application of acetylcholine to the auricular surface. In the calf the interval between its application and the start of fibrillation is greater, the fibrillation lasts longer than in dogs. Typical fibrillary waves occasionally may be present in the tracing taken on the surface of the right auricle but absent in the tracing from inside the left auricle.

**The electrogram of the fish heart.** By BRUNO KISCH, New York, N. Y. *From the Marine Biological Laboratory, Woods Hole, Mass*. Electrograms were taken from the surface and from the inside of the heart of Bone Fish and of Selachians with semunipolar leads. The voltage of electrograms registered in this way has the same range as that of dogs and rabbits. The electrogram of the auricle as well as of the ventricle shows a QRS complex, a T and sometimes a following U-wave. The duration of the QRS is normally 0.04 to 0.06 seconds but the QT distance has a long duration (0.4-0.8 sec). The statement of the literature that in the fish's heart a  $\nu$  conduction as a rule is slower and less good than v-a conduction could not be corroborated. The spread of the excitation wave starts synchronously on the inside and outside of the ventricle. The apex of the ventricle is activated later than the base, but the activity of the base lasts normally longer than that of the apex.

The bulbus cordis of Selachians can become pacemakers of the heart and its electric activity can be registered. The one ventricle of fish is anatomically incompletely subdivided into a left and right apartment. The electrograms taken in each of these compartments may differ from each other remarkably. Potassium Chloride increases the rate of the actual pacemaker and transforms potential pacemakers into actual ones. Acetylcholinechloride and Mecholyl applied to the auricle slowed down the heart rate, but did not start auricular fibrillation.

**Variations in skin temperatures of the feet and hands and the onset of sleep.** N. KIFITMAN, A. RAMSAROOP (by invitation), and T. ENGLMANN (by invitation). *Dept. of Physiology, Univ. of Chicago, Ill.* The sudden steep rise in the temperature of the feet one or two hours before (sometimes immediately before or after) the onset of sleep has been interpreted as evidence of peripheral vasodilation due to a decrease in sympathetic activity, manifesting a "vegetative preparedness for sleep." Our observations of skin temperatures of the toes, soles, and legs, as well as the fingers, palms and forearms (or cheek), under a variety of conditions, while confirming the reported facts concerning the feet, do not support the interpretation given to them. In general, skin temperature fluctuations in the toes are greatest, in the soles, intermediate, and in the calves, least. The same differential applies to the corresponding skin areas in the upper extremities, the absolute changes, however, being smaller. Following a meal, if there is a rise in oral temperature, there is a concomitant increase in toe and finger skin temperatures. Muscular relaxation, when it leads to a lowering of the body temperature, is usually accompanied by a drop in finger temperature and a simultaneous rise in toe temperature. In the pre sleep hours of the evening there is thus a combination of two toe skin-temperature raising influences: meal and muscular relaxation. It therefore seems that the feet, especially the toes, exhibit vasomotor changes that are not characteristic of the skin as a whole, not even of the skin of the extremities, and changes in foot temperatures cannot be looked upon as variations in general sympathetic activity.

**On naturally occurring porphyrins in the root nodules of leguminous plants.** HEINRICH KLÜVER. *The Division of the Biological Sciences, University of Chicago.* The fluorescence spectrum of the root nodules of various leguminous plants containing red or brown pigments exhibits an emission band at about 620 m $\mu$ . The porphyrin which we have extracted from the nodules of the soybean has the characteristics of a coproporphyrin. The spectrochemical evidence has been derived from data on solubility, HCl number, and mea-

surements of the fluorescence spectra of the free porphyrin and its methyl ester in various solvents. Previous work (Kubo, Keilin, Virtanen) has established the occurrence of hemoglobin in the root nodules of every leguminous plant so far examined. It becomes possible, therefore, to study a porphyrin-hemoglobin system in the absence of numerous factors influencing or complicating the relations between porphyrins and hemoglobin in the animal organism. Our preliminary studies of root nodules of the red kidney bean have revealed the presence of large amounts of a porphyrin which spectroscopically is practically identical with coproporphyrin, but which in other respects behaves like the Waldenström type of uroporphyrin. Future research will be necessary to determine whether the legporphyrins include a hitherto undescribed porphyrin. The physiological role of the porphyrins occurring in various organisms is not exactly known. The question must be raised whether the porphyrins present in the root nodules are directly or indirectly involved in the process of symbiotic nitrogen fixation, particularly in the earlier stages. The use of isotope methods for future investigations of this problem is suggested.

**A comparison of the effect of testosterone propionate and "growth hormone" on the body and organ weights and composition and the arginase and phosphatases of the kidney and liver of the castrated male mouse.** CHARLES D. KOCHAKIAN and CONSTANCE E. STETTER (by invitation). *Department of Physiology and Vetal Economics, University of Rochester, Rochester 7, New York.* White male mice were castrated at 17-19 grams body weight and approximately one month later, separate groups were treated for 10, 20 and 34 days as follows: a) injected daily with 1 rat growth unit of anterior pituitary growth hormone (Parke, Davis & Co.); b) implanted subcutaneously with a 14 to 15 mg pellet of testosterone propionate; and c) both treatments simultaneously. The mice treated with the growth hormone preparation showed approximately the same increase in body weight as those treated with the androgen pellet. When the two hormones were administered simultaneously there was a summation effect on the increase in body weight. The growth hormone increased the kidney and liver in proportion to the effect on body weight. The androgen produced the well known effects on the organs. The kidney weight was increased about three times that produced by the growth hormone. There was no summation of these responses when the two hormones were administered simultaneously. Both hormones increased the total amount of protein and water in the carcass and the organs and decreased the fat of the carcass. The increased amount of protein

was similar for both hormones except that the androgen diverted a large portion, 15-20 percent, to the seminal vesicles and prostates. The growth hormone caused a slightly greater decrease in water content and a much greater decrease in carcass fat. The simultaneous administration of the two hormones caused a summation of these effects. The androgen produced the expected changes in the enzymes of the kidney. The growth hormone increased all of the enzymes in proportion to the increase in mass of the organ. When the two hormones were given simultaneously there was a remarkably smaller increase in the arginase activity of the kidney, the phosphatases were not affected. The concentrations of enzyme activities in the liver were not affected by any of the treatments.

**Acute pulmonary edema produced by ammonium salts.** HAROLD KOENIG and RUTH KOENIG (introduced by W F WINDLE) *Anatomical Laboratories, University of Pennsylvania School of Medicine*. During the course of investigation of another problem, in which guinea pigs were administered ammonium chloride daily by gavage to produce chronic acidosis, it was noted that animals occasionally succumbed to an acute pulmonary edema. The characteristic sequence of events transpires during a period of fifteen to sixty minutes following the administration of 0.06 to 0.12 gm of ammonium chloride per 100 gm of body weight. The subcutaneous, intramuscular, intraperitoneal, intravenous and enteric routes of administration are all effective. Following a period of dyspnea, coma, muscle fasciculations and generalized clonic and tonic convulsions supervene. A serous or serosanguineous fluid usually exudes from the nostrils before exitus. The lungs are typically swollen and present areas of gross subpleural hemorrhage and congestion. Copious edema fluid can be expressed from the cut surface of the lung. The right heart and entering veins are greatly distended. Other ammonium salts in equivalent amounts in respect to the ammonium ion will produce pulmonary edema. Rats and cats also are susceptible. Bilateral cervical vagotomy, atropinization, and barbiturate anesthesia do not prevent the pulmonary edema although they occasionally alter the typical pattern of events. Analysis of edema fluid from one cat revealed it to be a transudate with no protein, indicating that an increase in pulmonary capillary permeability is not the prime factor producing this edema. However, the fluid is blood-tinged in many cases and probably contains serum proteins that leak out of the capillaries after anoxia sets in.

**Secretion of mucin in response to sham feeding and histamine stimulation.** S A KOMAROV, HARRY SHAY, HERMAN SINFY (by invitation) *Fels Re-*

*search Institute, Temple University, School of Medicine*. Although it has been long recognized that mucin in solution is one of the principal constituents of acid gastric juice, (Babkin, B P, *Am J Surg*, 7, 498 (1929), *Canad Med Assn J* 28, 134 (1931), Webster, D R, and Komarov, S A, *J Biol Chem*, 96, 133 (1932), Komarov, S A, *J Biol Chem*, 109, 177 (1935)) the mechanism of its secretion is little understood largely due to the lack of reliable quantitative methods for determination of this substance. With the aid of a newly developed method for mucin estimation, (S A Komarov, H Siple, and H Shay, *Federation Proceedings*, Vol 6, No 1, March, 1947) it has been possible to compare the secretion of mucin in addition to pepsin, acid and protein nitrogen under various conditions of gastric stimulation. Experiments performed on three dogs with gastric fistulae and oesophagotomy demonstrated conclusively that sham feeding stimulates the secretion of mucin as effectively as it does that of acid and pepsin. Furthermore, an analysis of the curves for concentration and output of the major constituents shows each of them to behave independently, an indication that the respective sets of secretory cells respond in a selective manner to the same stimulus transmitted through the vagus nerves. Experiments with histamine showed that the parietal cells alone are stimulated by this substance. The output of mucin remained constant at the level of resting stomach secretion, while pepsin secretion in many experiments was actually inhibited.

**Dermatome autonomic activity in relation to segmental motor reflex threshold.** IRVIN M KORR and MARTIN J GOLDSTEIN (by invitation) *Still Memorial Research Trust, Kirksville, Missouri*. Enduring differences in segmental reflex thresholds involving the spinal extensor motoneurons have been demonstrated in man (Denslow, J *Neurophysiol* 7 207, 1944). The low threshold segments appear to be those in which a relatively large portion of the motoneurons are maintained in a state of facilitation due to chronic bombardment by impulses from segmentally related structures (Denslow, Korrr and Krems, *Amer J Physiol* 105 229, 1947). In the present investigation evidence has been obtained that the facilitation extends to the cells of the intermediolateral column in the corresponding segments since measurements of electrical skin resistance indicate segmental differences in sweat gland activity which are related to the motor reflex thresholds. Electrical conductivity of the skin of the back was measured in our subjects by a convenient modification of the dermohmmeter. Under the conditions of our experiments most of the skin of the back has a resistance of 5,000,000 ohms or more. However, portions of dermatomes, and oc-

asionally entire dermatomes, related to segments with reduced thresholds have markedly reduced electrical resistance, often as low as 20,000 ohms. The largest, most constant and most reproducible differences in skin conductivity, related to segmental motor reflex thresholds, are found in the midline, over or near the vertebrae. Areas with reduced resistance are often hyperesthetic and some may have the characteristics of trigger areas. It is concluded that in segments with chronically reduced motor reflex thresholds, at least some of the preganglionic sympathetic neurons of the same segments are also maintained in a state of facilitation.

**Influence of unilateral adrenalectomy and sympathectomy on homolateral renal function** J. P. KRISS, P. H. FUTCHLER and M. L. GOLDMAN (introduced by H. A. SCHROEDER) *Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Missouri*. In 1914 Cow reported that unilateral adrenalectomy caused increased excretion of urine by the homolateral kidney, he also described direct vascular connections between adrenal and kidney. In 1919 Marshall and Kolls reported that changes in renal function following unilateral adrenalectomy were occasioned by interruption of autonomic nerves during adrenalectomy.

The excretion of water, chloride, mannitol, and sodium para aminohippurate was studied in dogs under nembutal anesthesia with both ureters catheterized. The excretion of all these substances by the left kidney exceeded that by the right kidney in 3 dogs several weeks after left adrenalectomy and in 1 dog immediately after left adrenalectomy. Increased excretion of these substances by the left kidney was also noted in 2 dogs with intact adrenals several weeks after left supradiaphragmatic sympathectomy and in 1 dog with intact adrenals immediately after the same operation. No similar difference in function of the 2 kidneys was observed in 2 dogs several weeks after bilateral supradiaphragmatic sympathectomy and left adrenalectomy.

Our results support the conclusion that unilateral sympathectomy and unilateral adrenalectomy produce the same effect upon those aspects of renal function which we investigated. In most, but not all, instances, the increase in glomerular filtration rate (mannitol clearance) sufficed as a possible explanation for the increased excretion of water and chloride by the kidney homolateral to the site of the operation.

**The adrenalin sensitivity of the denervated dog kidney** W. G. KUBICEK, R. B. HARVEY and F. J. KORTKE *University of Minnesota, Minneapolis*. Renal plasma flow (RPF), glomerular filtration (GF) and filtration fraction (FF) were determined by means of para aminohippurate

and creatinine clearance respectively. After control values were obtained epinephrine hydrochloride (Winthrop) was administered by a continuous intravenous infusion at a rate sufficient to elevate the diastolic blood pressure approximately 25 mm Hg. In most instances 4 to 6 mgm of adrenalin per hour produced the desired results. Nausea and vomiting occurred in some cases if the adrenalin infusion rate became excessive. In dogs with normal renal nerves no significant change was noted in RPF, GF or FF. Dogs with denervated kidneys showed a marked reduction (40 to 50 per cent) in RPF and GF with no significant change in FF. The data indicate that the dog with normal renal innervation can maintain a constant renal function during a large increase in arterial blood pressure produced by the above procedure while the increased sensitivity to adrenalin in the denervated kidney produces a vasoconstriction of greater proportion than the increase in blood pressure. This factor may be of importance in connection with sympathectomy in man.

**Effect of sinus gland extract on the rate of phosphorylation of arginine** LOIS KURTZ (introduced by IRWIN SIZER) *Arnold Biological Laboratory, Brown University*. Many types of investigation have demonstrated the endocrine nature of the crustacean sinus gland. An investigation of the effect of crayfish sinus gland extract on the rate of transfer of phosphate from adenosine triphosphate to phosphoarginine by muscle homogenate was made. Homogenates prepared from the abdominal muscles of crayfish were pre incubated with an aqueous extract of sinus gland, then incubated with a substrate containing arginine and adenosine triphosphate in carbonate buffer at pH 9. Magnesium chloride and potassium chloride were also added. The control experiments contained all components except sinus gland extract. An acceleration of phosphate transfer from adenosine triphosphate to arginine of the order of two to four times occurred in the presence of sinus gland extract.

**Effect of lactogenic hormone on estrogen response of immature rats** ANNETTE LABELLE (by invitation) and RICHARD TISLOW *Biological Research Laboratories, Schering Corporation, Bloomfield, New Jersey*. When lactogenic hormone (prolactin) was administered to immature rats in which corpora lutea had been induced by the administration of human chorionic gonadotrophin, no vaginal cornification was produced by an otherwise effective dose of estradiol benzoate.

**A study of the ankle jerk in myxedema** EDWARD H. LAMBLERT, LUIS O. MEDEROS (by invitation) and MAVIS P. KELSEY (by invitation) *Sections on Physiology and Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota*. The strength and duration of the tap on the tendon, muscle reaction potential and muscular tension have been re-

corded simultaneously in studying the ankle jerk of 20 normal subjects and 25 patients with myxedema. The duration of the contraction and relaxation phases of the muscle response were 0.14 (0.11-0.19) and 0.33 (0.19-0.49) seconds, respectively, in normal subjects (BMR +4 to -12), but were 0.23 (0.19-0.28) and 0.74 (0.43-1.09) seconds, respectively, in myxedema (BMR -16 to -39). The duration of contraction was generally inversely proportional to the BMR. It decreased as BMR increased in hypothyroid patients given desiccated thyroid. It increased as BMR decreased in patients developing myxedema following administration of radioactive iodine. The total latent period (0.035 seconds) and the contour and duration of the muscle action potential were not significantly different in myxedema and normal subjects. However, the duration of a single muscle twitch following direct electrical stimulation was greater in myxedema than in normal subjects. The temperature of the muscle in myxedema was 0.5-1.0°C lower than that in normal subjects, but it was necessary to reduce the temperature of normal muscle approximately 10°C to slow its response to the degree found in myxedema. Furthermore, the ankle jerk in myxedema differed from that of normal muscle cooled to 25-28°C in that (1) the action potential was not prolonged as it was in cooled normal muscle, and (2) a "shoulder" was prominent in the relaxation phase of the response of myxedema muscle, but not in cooled muscle.

#### Studies on the nutritional effects of heated fats

A. LANE (by invitation) and A. C. ILLI, *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago*. The work of several investigators suggests that fats heated at high temperatures are nutritionally harmful and may be carcinogenic. The following experiment was designed to study the effect of heated fat as compared to unheated fat on the nutrition of rats. Two diets composed of a homogeneous mixture of bread, milk and lard which in one diet was heated to 350°C for thirty minutes and unheated in the other diet were fed to young adult white rats. Four synthetic diets, two adequate for rat nutrition, the fat content of one being heated (350°C for 30 min) and two diets high in fat (43%) and low in protein (8%) one of which contained heated fat were fed to four groups of rats. Weekly body weight and daily food intake were recorded, and at intervals animals from each group were killed, tissues taken for histological study and total fat determinations run on livers. There is little difference in food intake between rats on unheated and heated fat with the bread, milk and lard diets, both groups show skin lesions and eye signs suggestive of vitamin A deficiency. On the synthetic diet, the group eating the "adequate heated fat diet" ate slightly less and body weight curve is

slightly below the "unheated fat group". In the group eating high fat which has been heated, the food intake and body weight curve are below the group eating the same diet with unheated fat content. These studies are being continued.

**Cyclic changes in the respiratory center** M. G. LARRABLE and ROBERT HODES, *Johnson Foundation, University of Pennsylvania*. Progressive changes in the respiratory centers, occurring during the respiratory cycle, were tested by afferent impulses over the superior laryngeal nerve. In several experiments the minimum numbers of afferent volleys required to stop inspiration were determined at various times during inspiration. Thus measured, the threshold for stopping inspiration fell linearly from early in inspiration to reach zero at the end of inspiration. Thus changes which normally terminate inspiration develop progressively throughout inspiration, and follow a simple time course. In other experiments constant numbers of afferent volleys were initiated at various times during expiration, the delay caused in the next inspiration was observed. The maximum delay resulted from afferent volleys initiated just before the normal start of inspiration. Delays due to afferent volleys earlier in expiration were expressed as percent of the maximum delay, in animals breathing at widely different rates. Delays due to afferent volleys one second before the end of expiration were smaller fractions of the maximum, the shorter the animal's natural expiratory pause. Delays due to afferent volleys at the start of expiration were always between 39 and 47% of the maximum, with no consistent change with duration of expiration. This constant reduction in inhibitory effect, during a time equal to one expiration, may be explained by assuming equal rates of decay for a) the central effect produced by afferent impulses and b) for inhibitory effects which normally develop during inspiration and subside during expiration. This further suggests some mechanism in common for the two effects.

**Estimation of the rate of antidiuretic hormone secretion in normal man** HENRY D. LAUSON, HOWARD A. EDER (by invitation), FRANCIS P. CHINARD (by invitation), GEORGE C. COTZIAS (by invitation) and ROGER L. GREIF (by invitation), *Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.* From studies on dogs with experimental diabetes insipidus, Shannon (*J. Exp. Med.* 76: 387, 1942) concluded that the normal rate of secretion of antidiuretic hormone in intact 10 to 15 kg dogs is equivalent to the intravenous administration of from 1 to 5 milliunits of pituitrin per hour. In order to make the same kind of estimate in a normal 70 kg man, the endogenous secretion of antidiuretic hormone was inhibited by rapidly drinking 1500 ml of water and the inhibition maintained for 8 hours by drink-



ing water in volumes equal to the urine output. After a 2 hour control period of steady maximal diuresis, commercial Pitressin was infused intravenously at the constant rates of 7.5, 16.1 and 50 millunits per hour during 3 consecutive 2 hour periods. On another day under the same regimen, 333 millunits per hour were administered for 2½ hours. The endogenous creatinine U/P ratio (urine concentration/plasma concentration) was taken as the index of renal tubular water reabsorption. Assuming that after 1½ hours of constant infusion, equilibrium has been attained between the rate of Pitressin inflow and the rate of its removal from the blood, the U/P ratio during the next half hour measures the renal response to the given infusion rate. For the rates of 0, 7.5, 16.4, 50 and 333 millunits per hour, the following U/P ratios were found at equilibrium: 7.2, 80.8, 138, 183 and 131, respectively. Per kg body weight, these results are essentially the same as obtained by Shannon in dogs with diabetes insipidus.

**High protein diets and testosterone propionate as related to plasma and liver proteins in rats.** JAMES H. LEATHEN, *Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey*. The influence of a high protein diet on adrenal size and function has been studied in adult and young rats over a 20-25 day period. Diets of 78% casein or lactalbumin were the high protein diets used whereas diets of 18% protein or fox chow were considered as control diets. Body weight increase was retarded by the high protein diets, an effect which was more pronounced in adult rats by the use of lactalbumin. A modest hypertrophy of the adrenals was noted and a significant increase in kidney and liver weight was obtained. If the plasma protein concentrations are used as a criterion of adrenal function then the modest adrenal hypertrophy noted did not alter function. This is indicated by the fact that total plasma protein, albumin and globulin concentrations were not influenced by diet although NPN was increased by a high protein diet. The increase in liver size, however, was accompanied by an increase in total liver protein in the body and an increase in protein percentage of the dry weight. Water content of the liver was not changed. Since testosterone propionate is known to cause urinary  $N_2$  retention, the effect of this androgen in combination with varied diets was studied. Testosterone propionate (Perandren, Ciba) was administered three times weekly in dosages of 1.0 mg. This amount of androgen did not influence organ weight, plasma protein concentrations or liver protein content.

**The effect of intravenous sodium cyanide on the electrocardiogram.** ALFRED LEIMDORFER, *Department of Psychiatry, University of Illinois*. Intravenous injection of NaCN (0.2-0.8 mg/kg) causes prompt, brief, reflex slowing of the heart in

cat, monkey and man. In the cat this depends on the carotid bodies and vagi. During the first slowing, high take off waves, similar to those seen in the acute stages of coronary occlusion, appeared in the cat alone. A second slowing, with increased T P and P-R intervals, follows. With toxic doses in the cat (0.9-2.1 mg/kg) this passes into extreme bradycardia and arrhythmia. There also appeared in cat, monkey and man increased amplitude of T waves and elevation of S-T segment. These and other typical anoxic changes varied with the dose of NaCN. With the largest doses in cats signs of myocardial failure appeared in the form of severe arrhythmias, a-v and intraventricular blocks, extreme low voltage, ventricular flutter and fibrillation.

**Electroencephalographic analysis of action of amidone, morphine and strychnine on the central nervous system.** ALFRED LEIMDORFER, *Department of Pharmacology, University of Illinois, College of Medicine, Chicago*. In cats, amidone HCl induces changes in the electroencephalogram (EEG) (of the parietal and occipital lobes) with 3 mgm/kgm of body weight i.v., there appear transitory diphasic spikes alternating with a decrease in frequency. After the i.v. injections of larger doses (18 mgm per kgm over a period of 6½ hours), transitory, high voltage, high frequency discharges, indicative of seizures, occur. Following the initial change, the control activity returns. After the i.v. injection of still larger doses (22.5 mgm per kgm within 6½ hrs), the electrical activity of the brain is abolished. No changes in the electrospinogram are obtained. In cats, morphine sulfate (5 mgm/kgm, subcut), produces a slight transient increase in the frequency of the EEG. The i.v. injection of large doses (40 mgm per kgm), induces a great decrease in the frequency and voltage of the EEG, after the additional i.v. injection of 40 mgm per kgm, about an hour later, the brain waves disappear. The injection of morphine rarely produces the appearance of diphasic spikes in the EEG and occasionally the occurrences of tetanic waves in the electrospinogram. In cats, strychnine sulfate (0.5 mgm/kgm subcut or i.v.) produces a burst of tetanus waves in the electrospinogram. The i.v. injection of large doses (2.5 mgm/kgm) evokes characteristic diphasic spikes in the EEG. Section of the spinal cord shows that tetanic waves arising from different parts of the spinal cord and the spikes in the EEG are independent in origin.

**Auditory and visual areas of the cerebral cortex of the rat.** D. H. LEMESSURIER (introduced by C. N. WOOLSEY), *Dept. of Physiology, Johns Hopkins University, School of Medicine, Baltimore 5, Md*. The evoked potential method has been used to define the auditory and the visual areas of the rat's cerebral cortex. The potential changes produced

in the cortex either by photic stimulation of the eye or by click stimulation of the ear are very large and may range from 1500 to 2500 microvolts under prolonged pentobarbital anesthesia. The main auditory area, centered over Krieg's area 41, is approximately 4 mm wide dorsoventrally and about 5 mm long antero posteriorly. There is also another area which can be activated by sound. This apparently corresponds to Tunturi's third auditory area. It is closely related to the face subdivision of somatic area II (see Woolsey, and LeMessurier, this issue). The visual area corresponds approximately to Krieg's areas 17, 18 and 18a. Both eyes project to this area, but the responses evoked by stimulation of the ipsilateral eye are large only in the central part of the field activated by the contralateral eye. There is a slight degree of overlap between the visual and auditory areas posteriorly. Both areas overlap somewhat the adjacent portions of the somatic areas. Whether these overlaps mean anatomical intermingling or result from the method has not been decided. In any case there is no cortex between the various receiving areas which could constitute any appreciable associational cortex. The nature of the overlap encountered does not suggest that associative functions are subserved by the overlap.

**The secondary blood pressure rise and tachycardia occurring after the injection of epinephrine.** R. LEVEL (introduced by S. ROBBARD). *From the Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Illinois.* We have observed that during the course of the primary pressor action of large doses of intravenous epinephrine, injected in trained unanesthetized dogs, a secondary rise in blood pressure of the order of 75/75 mm Hg above the primary peak may occur. The entire secondary rise in pressure occurs within a beat or two, and it is followed immediately by a paroxysm of ventricular tachycardia which may persist for a few seconds or for as much as two minutes. The incidence of the occurrence and the duration of this effect seen with various doses of epinephrine is given in the table.

| Epinephrine injected | Animals with secondary rise | Average rise | Duration of tachycardia |
|----------------------|-----------------------------|--------------|-------------------------|
| mg                   | %                           | mm Hg        | sec                     |
| 0.01                 | 0                           | 0            | 0                       |
| 0.1                  | 22                          | 63           | 32                      |
| 0.5                  | 50                          | 66           | 32                      |
| 1.0                  | 75                          | 96           | 118                     |
| 2.0                  | 80                          | 78           | 135                     |

The effect is inhibited by atropine, dibenamine or by pentobarbital anesthesia. It is partially inhibited by morphine.

**Chemical and physiological changes during water tolerance tests in patients with cirrhosis.**

STEPHEN LESLIE (by invitation), GEORGE H. STUECK, JR. (by invitation), HAROLD M. SHORR (by invitation) and ELAINE P. RALLI. *Department of Medicine, New York University College of Medicine.* Water tolerance tests were done in patients with cirrhosis of the liver, both with and without ascites, and in normal subjects. The tests were done by administering 1500 ml of tapwater orally. The plasma volume was determined prior to the test. Blood and urine samples were taken at intervals during the tests. The hematocrit, total protein, albumin, globulin, sodium and chloride were determined on the serum. The rate of urine flow was accurately determined by collecting the urine by catheter and the specific gravity and the chloride content were done on the urine samples. To rule out the effect of intra-abdominal pressure on the rate of urine flow, some tests were done on the same patient both before and after a paracentesis. In other patients repeated tests were done during periods when ascites was present and when ascitic fluid had ceased to reaccumulate. The results showed that in patients with cirrhosis of the liver there is a marked retardation of the rate of urine flow which occurred even when there was no evidence of ascites. The plasma volume as determined by the dye method was increased in all of the patients. During the test the chloride content of the serum fell regularly. The other fractions determined showed certain variations which will be presented.

**The path of suppression in the spinal grey matter.** JEROME Y. LETTVIN (introduced by W. S. McCulloch). *Department of Psychiatry, University of Illinois.* In the cat's spinal cord, soaked in nembutal in situ to prevent transynaptic activity, impulses evoked by electrical stimulation of the bulbar reticular suppressor mechanism were recorded on one occasion from the medial internuncial pool of the ventral horn, and regularly from the lateral portion of the intermediate pool of internuncials. Even without soaking in nembutal, they have never been found in any other position in the spinal grey. Stimulation in this lateral portion of the intermediate internuncial pool with 30 micro amperes completely inhibited the knee jerk. Pick up and stimulation of cord were through concentric bipolar microelectrodes separated less than half a millimeter. Their positions were histologically determined by Dr. R. S. Snider.

**The disposal of intravenously administered amino acids by normal, hepatectomized, depancreatized and hyperthyroid dogs.** R. LEVINE and N. G. SCHNEEBERG (by invitation). *Department of Metabolism and Endocrinology, Research Institute, Michael Reese Hospital, Chicago, Illinois.* Dogs under morphine sedation, were injected intravenously with varying amounts of amino acids at a constant rate for 2 hours. Amino acid and urea

levels were followed in the blood and urine for 6-8 hours, beginning with a control period of one hour. During the experimental period the total extra nitrogen excretion amounted to 8-12% of the amount administered. 88-92% of the amino acids injected were returned. Neither the liver nor the pancreas seem to be necessary during this phase of amino acid assimilation. Pretreatment with thyroxine did not increase the N excretion during this period over the values in the normal animal.

**Quantitative examination of sensibility in peripheral nerve injuries.** F. H. LARSEN, *The Peripheral Nerve Study Center, Hosp. of the Univ. of Pennsylvania*. Threshold examinations for returning sensitivity following nerve injuries by means of v. Frey's hairs and Heed's algometer are satisfactory as long as improvement is progressing. However, the results become equivocal when no improvement of threshold values is apparent three months after the last examination. The conclusion might be drawn that the nerve had met an obstacle and should be explored surgically. Yet, threshold determination is only one parameter of functional return of sensitivity. The number of touch and pain fibers which at a given time have made contact with their peripheral end organs is another. Careful sensory examination requires 1) counting of the number of touch and/or pain points per cm<sup>2</sup>, present in a previously anesthetic and analgetic area, and 2) their threshold in terms of grams of pressure. To give a practical example. In the analgetic area of an injured radial nerve on the back of the hand one pain point p/cm<sup>2</sup> was discovered on schedule after nerve suture. These points felt pin prick under 8 g of pressure. Three months later, the threshold was still 8 g, and the patient seemed to be doomed for another operation. Fortunately, count of the pain points showed that their number had increased from 1/cm<sup>2</sup> to 20/cm<sup>2</sup> indicating that many more nerve fibers had arrived in the skin and made contact with their end organs, although their maturation had lagged behind. The operation was called off and the continued progress of recovery justified the decision.

**Nature of increased serum  $\beta$ -globulin content in malignant hypertension and diabetic retinopathy.** LENA A. LEWIS and IRVINE H. PAGE, *Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio*. A consistent, absolute and relative increase of plasma  $\beta$ -globulin concentration has been found by electrophoresis of samples from patients suffering from malignant hypertension (Lewis and Page) and from diabetic retinopathy (Lewis, McCullagh and Schneider). Since this abnormality of electrophoretic pattern might arise from an increase in lipid loosely bound to  $\beta$ -globulin and migrating therewith, post-absorptive samples of serum from such patients were re-examined after cold acetone and ether extraction (Blum). Electro-

phoresis was done using phosphate buffer pH 7.8, ionic strength 0.16M. The total protein concentrations were above the lower limits of normal.

As was expected, extraction decreased the area of the  $\beta$  globulin peak. However, the relative concentration of  $\beta$  globulin after extraction was consistently greater in sera from cases of malignant hypertension and diabetic retinopathy than in normal sera after or even before extraction. In lipid extracted sera from normal subjects, the  $\beta$  globulin area represents 12.0 to 15.4 (mean 13.6) per cent of the total protein shadow, while in patients suffering from malignant hypertension the range of  $\beta$  globulin after extraction ranged from 16.1 to 32.8 (mean 20.2) per cent of total. These forms of cardiovascular disease are therefore shown to be associated with an increase in serum  $\beta$  globulin content which is not due to loosely bound lipid. Although increased thymol turbidity has been related to high concentrations of  $\beta$  globulin in liver disease, thymol turbidity was not increased in the form of hyper  $\beta$  globulinemia under study.

**Adenosinetriphosphatase (ATP-ase) in nerve.** B. LIBET, *Dept. of Physiology, The University of Chicago*. It is conceivable that a basic feature of nerve impulse conduction, the permeability change in the membrane, depends upon a structural change in certain membrane proteins. If the latter resemble the myosin system of muscle, they should be associated with ATP-ase activity. To test this hypothesis, measurements were made of the ATP-ase activity of the axoplasm, extruded from cleaned giant axons of the squid *Loligo pealii*, and of the remaining sheath portion. With the tissue homogenized in 0.55 M KCl, and the activity measured in 0.55 M KCl, 0.003 M  $\text{CaCl}_2$ , veronal buffer (pH = 7.4) and 0.003 M ATP, axoplasm splits phosphate from ATP at the rate of about 0.2 micrograms P/mg wet weight in 30 minutes at about 26°C, while the average figure for the sheath is 19, about 100 times as much. Not only is the ATP-ase activity of the nerve fiber almost exclusively confined to the sheath portion, but the latter has an activity even greater than that of squid muscle. Contrary to the case of cholinesterase (D. Nachmansohn & B. Meyerhof, *J. Neurophysiol.*, 1941, 5: 348-361), ATP-ase is not more concentrated in the optic ganglion than in the axon sheath. Nerve ATP-ase also resembles vertebrate muscle ATP-ase in its substrate specificity and in its sensitivity to  $\text{Ca}^{++}$  concentration, though it is considerably less sensitive to sulphydryl poisons. More purified preparations of ATP-ase from nerve tissue will permit a fuller comparison of the physical and chemical properties of this enzyme with the myosin-ATP-ase system.

**Effect of stretch on membrane potential in frog muscle.** G. LING (introduced by R. W. GERARD), *Dept. of Physiology, The University of Chicago*. The previously described technique (Graham and

Gerard, J C C P 28 99, 1946) for measuring the membrane potential of a single muscle fiber by inserting a micro electrode has been improved with a capillary electrode of smaller diameter (app  $1\ \mu$  i d,  $3\ \mu$  o d, measured  $5\ \mu$  away from the tip) and gentle taper, the single fiber resting potential of frog sartorius is found to be higher and more constant For nearly 200 fibers from 20 muscles, the average is  $77.5 \pm 4.7$  mv, maximum, 90 mv, minimum, 60 mv Stretching of striated muscle has been reported to effect heat production, respiration, pH, excitability, action and demarcation potential, etc It was anticipated that stretch would alter the membrane potential Measurements on many fibres from some twenty muscles, however, showed no significant differences between values obtained obtained before, during or after stretching muscles to length between 115 and 170% of the unstretched value This is true even though muscles stretched to 170% remain some 10% longer than normal when released

**Effects of protein and fluid consumption upon plasma volume and circulating protein in the rat** RICHARD W LIPPMAN, (introduced by T ADDIS) *Department of Medicine, Stanford University School of Medicine* Rats were fed upon diets which varied from 3% to 77% in protein content, and contained variable quantities of fluid and salt At the end of the experimental periods, 17 hours and 7 days, blood and plasma volumes were determined by a dye method, and total serum protein was determined by the biuret method Total circulating protein was calculated from the measured data Results were compared with normal for rats of the same size, as previously reported, in order to avoid errors inherent in surface area calculations Changes in blood and plasma volumes were in no instance striking Fluid diet with added salt produced a transient 10% increase in plasma volume Blood volumes were increased only in groups which lost weight during the experiment, and then in such proportion as would be expected from the relative stability of the total erythrocyte mass over short periods The high protein diet produced only slight increases in blood and plasma volumes, and only 6% increase in total circulating protein, at the end of 7 days All protein deficient diets reduced total circulating protein 15-20% at the end of 7 days The apparent contradiction between our data and that of Metcalf, Favour and Stare (J Clin Invest 1945, 24, 82) is due to their mode of expression in terms of "unit" function per 100 sq cm, as the lines relating blood and plasma volumes and total circulating protein, to surface area in the normal rat do not intercept either axis at 0

**Two fractions of specific cholinesterase present in normal mouse brain** J MAXWELL LITTLE *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College,*

*Winston Salem, North Carolina* Mouse brain homogenate [NaHCO<sub>3</sub> buffer, pH 8.5] was centrifuged The supernatant had 15-20 per cent and the resuspended precipitate had 80-85 per cent of the cholinesterase activity of the uncentrifuged homogenate The supernatant from a distilled water homogenate [pH 6.8] contained 90-95 per cent of the uncentrifuged activity Adjustment of distilled water homogenate to pH 8.5 by NaOH diminished the supernatant activity due to enzyme destruction, varying the NaCl molarity up to 0.075M before centrifugation resulted in a shift of activity from supernatant to precipitate, beyond this there was no further change

The activity pattern of the supernatant and precipitate fractions was that reported for specific cholinesterase with the following substrates [0.015M] acetylcholine, acetyl- $\beta$ -methylcholine, propionylcholine, benzoylcholine and ethyl butyrate There was no significant difference in the activities of the two fractions with acetylcholine when tested at pH 6.5, 7.0, 7.5 and 8.0 [optimum pH 7.5] Incubating the supernatant and precipitate fractions obtained by centrifuging a distilled water homogenate made 0.15M with NaCl resulted in the following respective changes expressed as per cent of the activity of the unincubated fractions 60°, 15 minutes—0 per cent, 0 per cent, 53°, 15 minutes—86 per cent, 93 per cent, 30 minutes—80 per cent, 72 per cent, 45 minutes—78 per cent, 50 per cent, 60 minutes—76 per cent, 33 per cent Mouse brain contains two fractions of specific cholinesterase, one of which is precipitated from a water homogenate at a NaCl molarity of 0.075 or greater The two fractions differ in their heat labilities

**Action of antacids in the human stomach, Results with zirconium phosphate** A LITTMAN (by invitation) and M I GROSSMAN *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago* To study the effect of antacids on the volume of the gastric contents as well as on acid concentration a new technique was devised Following histamine injection and introduction of antacid into the stomach tube, aspirations were made by the method introduced by Bloomfield and Keefer for study of the gastric secretory response to the alcohol test meal The gastric contents were aspirated completely at 10 minute intervals, the volume measured, and all but a 10 cc sample promptly returned to the stomach The usual pH and "free" and "total" acid determinations were made on the samples The effects of a thixotropic aqueous suspension of zirconium phosphate and aluminum hydroxide gel were compared, using 100 cc volumes of each In patients having a large acid output following histamine in control experiments, aluminum gel was shown definitely to be superior

In patients having low acid response differences were small. It was thus shown that evaluation of the efficiency of gastric antacids by such a method requires consideration of the levels of acid output in control experiments as related to the dose of antacid used in the tests.

**"Cortical instability"** a study of frequency effects. ROBERT B. LIVINGSTON (by invitation) and JOHN F. FULTON, *Lab of Physiology, Yale University School of Medicine, New Haven, Conn.* T. Graham Brown and C. S. Sherrington (*Proc Roy Soc* 1912, B85: 250-277) and A. S. F. Levton and C. S. Sherrington (*Quart J Exper Physiol* 1917, 11: 135-222) called attention to the "instability" of single cortical points in the motor area. Repeated stimulation of a single point sometimes gave inhibition, sometimes facilitation, reversals of response were also encountered, i.e. the same point might give flexion of a digit at one time and extension the next. E. P. Bowdoin and Marion Hines (*Amer J Physiol* 1933, 106: 175-182) stimulated a cat and two macaques and reported evidence that the "instability" of the motor cortex was related to the frequency and intensity of stimulus. P. Bailey and G. von Bonin (*Trans Amer Neurol Assn* 1946, 89-93) noted that no facial movements could be obtained from the monkey face area when stimulated at very low frequencies and that at these frequencies only movements of the tongue and vocal cords were evoked. O. Wyss (*J Neurophysiol* 1947, 10: 315-320) provoked different central responses by stimulation of the vagus nerve in the monkey at different frequencies. F. Nulsen (personal communication) observed that alteration of frequency of stimulation of the anterior cerebellum changes response of cerebrally induced movements from inhibition to facilitation as the frequency scale is ascended. We have observed that frequency of stimulus is an important factor in eliciting autonomic responses to stimulation of the frontal areas in monkeys and man. A systematic study of certain cortical areas has been undertaken to learn the frequency range for different responses in relation to the different functional modalities of the particular cortical area.

**Potentials of dorsal roots and related phenomena.** DAVID P. C. LLOYD and A. K. MCINTIRE (by invitation) *Laboratories of The Rockefeller Institute for Medical Research, New York.* Potentials have been recorded in stimulated and adjacent dorsal roots. In an adjacent root, there are five consecutive deflections, only the last of which has achieved recognition as the so called dorsal root potential. The first three (D R I, II and III) are an inverse electrotonic recording of the triphasic intramedullary spike potential of the travelling afferent impulses (negative, positive, negative at the central root lead). D R IV is positive, and overlapped by D R V, when cleared from it by

asphyxia. D R IV relaxes exponentially to half value within 3 msec. D R V is the familiar dorsal root negativity of Barron and Matthews. In a transmitting root, the spike potential is diphasic, followed by a negative potential of complex origin. The components have differential susceptibility to asphyxia. They are the after-potentials of the afferent fibers, a negative potential similar to the positive D R IV of adjacent roots, and a negative D R V. Since the central projections of afferent fibers are mutually parallel, an EMF generated by them must record electrotonically with opposite sign in transmitting and adjacent roots. D R IV, therefore, is referable to afferent fibers, and must be an electrotonic extension of the potential of terminating collaterals. D R V, of identical sign in transmitting and adjacent roots, must arise beyond the afferent fibers. The negative cord potential has two components, dissociable by asphyxia. The more resistant of these is temporally identical with D R IV. It may be accounted for by a flow of current from the patent afferent fibers to the active collaterals. Positive cord potential and D R V are temporally identical.

**Glomerular filtration rate in the adrenalectomized, salt fed rat.** W. D. LOTSPEICH (introduced by R. F. PITTS) *Department of Physiology, Syracuse University College of Medicine, Syracuse, New York.* Following clearance determinations in the intact animal, the kidney can be removed and isolated biochemical systems studied by tissue metabolism techniques. The rat is a suitable animal for this type of study. As a preliminary to such studies it was necessary to compare the rate of glomerular filtration in normal and adrenal insufficient rats. Twelve ad libitum fed controls, twelve adrenalectomized and six pair fed controls were studied. The creatinine clearance was used to measure glomerular filtration rate. After allowing all animals a three day acclimatization period on a "calf meal" diet, twelve rats were adrenalectomized. During the next two weeks records of body weight and food consumption were kept daily on all animals, and the adrenalectomized were allowed 1% sodium chloride to drink. Creatinine clearances were done during the third week when the adrenal insufficient animals were fully recovered and had established equality of body weight with their controls. It was found that glomerular filtration rate in the salt maintained adrenal insufficient rats was within the same limits as that of the normals. (Ad lib fed controls averaged 731 cc/100 gram/min, adrenalectomized averaged 901 cc/100 gram/min, and the pair fed controls averaged 840 cc/100 gram/min.)

**Heat loss and blood flow in the feet.** L. LOVELL (by invitation) and H. C. BAZLITZ *Department of Physiology, University of Pennsylvania Philadelphia, Pa.* Daily measurements of the heat loss of the foot,

blood flow and skin temperature have been made on two subjects living in a controlled temperature room for periods up to two weeks. Two conditions (approximately 33°C and 21°C) were used during the summer and again during the winter. During any single exposure to one temperature the only gradual change was in the temperature distribution along the foot. There were, however, considerable differences between three different periods of exposures to heat at different times of the year. The evaporative heat loss decreased after exposure to cold and there were associated changes in skin temperature, non evaporative heat loss and blood flow. It has been possible to estimate the amount of heat lost from the foot as a percentage of the heat loss of the entire body. The area of the foot amounts to about 5% of the body surface. From this area 3.5% of the heat was lost in the cold and 6.5% in the heat. It has been found that Newton's law of cooling does not apply to the foot. For an equal temperature difference between skin and air about twice as much heat was lost in the heat as in the cold. This can be attributed either to a roughening of the skin in the cold, or to the change in temperature distribution already mentioned, since convective heat loss is affected by the curvature of the surface.

**The effect of previous carbohydrate deprivation on the carbohydrate metabolism of isolated muscle.** KNUD LUNDBAEK (by invitation) and JAMES A. F. STEVENSON (by invitation) (Introduced by C. N. H. LONG) *Dept. of Physiological Chemistry and Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Following starvation or carbohydrate deprivation the administration of carbohydrate reveals an impaired sugar tolerance and a very small or no increase in the respiratory quotient. The previous carbohydrate intake alone, not the total caloric intake, is the important factor in producing this state. To investigate the capacity of the peripheral tissues to handle carbohydrate at this time the isolated diaphragm of the rat was used. For two to three weeks one group of adult female rats was given a carbohydrate free diet (80% fat, 20% protein by calories), and a second group a diet composed of 70% carbohydrate, 10% fat and 20% protein. Both diets contained adequate supplementary nutrients. At the end of this period the glucose uptake and glycogen synthesis were measured in the isolated diaphragm by incubation for three hours in a modified Krebs Henseleit solution containing 200 mg % glucose. There was no significant difference in the amount of glycogen synthesized. However, the glucose uptake of the diaphragms from the carbohydrate deprived rats was little more than half that of the diaphragms of the rats which had received a high carbohydrate diet.

**Concerning the nature of menstrual poison.** DAVID I. MACHT *Division of Pharmacology, Sinai Hospital, Baltimore, Maryland.* It is twenty five

years since the author published the first experimental proofs concerning the presence of a toxin in the blood of menstruating women. By means of quantitative plant-physiological experiments "menotoxin" was demonstrated in the blood, sweat, milk, tears, saliva and other secretions of such individuals. At that date the author concluded that menotoxin is a steroid closely related to oxycholesterol. Since then numerous hypotheses have been advanced as to its nature. In the present investigation the author tested by his phytopharmacological methods all such substances. The following bodies have been claimed as related to menotoxin: 1 various vasoconstricting substances 2 choline 3 di-methylamine 4 thyroxin 5 arsenicals 6 histamine 7 ascorbic acid 8 "necrosin." All these have been either prepared or secured by the writer and subjected to phytopharmacological tests. None of them were found to be phytotoxic to an extent to be regarded as "menotoxin." On the other hand, numerous experiments with female sex hormones and a large number of other steroids strengthen the original view of the author that menotoxin is related to cholesterol and oxycholesterol, and while a separate entity, is closely allied to the female sex hormones. Certain biophysical observations obtained with sterols irradiated with ultraviolet and roentgen rays corroborated this view.

**Thromboplastic effects of sulfa and penicillin combinations.** DAVID I. MACHT *Division of Pharmacology, Lab. of Sinai Hospital, Baltimore, Md.* The author has already described the powerful thromboplastic properties of penicillin, and its principal constituents (Science 71:302, 1930). Inasmuch as both sulfa drugs and penicillin are often administered together especially to surgical patients it is desirable to ascertain the effect of such combinations on blood coagulation. Experiments were made on rabbits and cats. Injections of sulfadiazine and sulfathiazole were made intraperitoneally or intravenously. Penicillin was administered either by vein or intramuscularly. Blood was secured either from carotid artery or by cardiac puncture. Neither sulfadiazine nor sulfathiazole affect coagulation time. Penicillin injections of either crystalline or amorphous variety quickly decrease coagulation time. This occurs both when the penicillin is given after the sulfa drug and when both are injected simultaneously. Too much emphasis can not be made on the importance of possible thrombo-embolic phenomena in patients receiving prolonged penicillin therapy, and the instituting of anticoagulant therapy.

**Changes in skin temperature and blood flow of hand following ingestion of certain amino acids.** MARTIN B. MACHT and ELIZABETH L. PHILLION (introduced by H. S. BELDING) *The Quartermaster Corps, Climatic Research Laboratory, Lawrence,*

**Mass** Skin and rectal temperatures, oxygen consumption, and blood flow through the hands of four healthy men were studied before and after oral administration of various amino acids. At an environmental temperature of 24°C ingestion of glycine, in amounts of 1, 2, 3, and 4 grams/10 lbs body weight, resulted in average hand skin temperature increases of 3.5°C, 4.8°C, 5.4°C, and 7.7°C, respectively, accompanied by similar, though less marked, increases in toe temperature and significant increases in blood flow through the hand as measured by venous occlusion plethysmography. Average maximum flows were 2.5 to 7 times the control flow. Increases in blood flow and hand skin temperature became apparent about 80 minutes after ingestion, being most marked approximately 180 minutes after ingestion. No significant changes in rectal temperature, or skin temperatures elsewhere were observed. At environmental temperatures of 18°C and 30°C, no significant changes in skin temperatures or peripheral blood flow occurred after glycine administration. At an environmental temperature of 24°C, ingestion of phenylalanine effected increases in hand and toe temperatures and blood flow through the hand similar to those observed with glycine, oral administration of histidine resulted in slight but significant increases in hand skin temperature and blood flow. Ingestion of glutamic acid, tyrosine, leucine, and methionine had no effect upon skin temperature or peripheral blood flow. Although five of the seven amino acids caused definite increases in oxygen consumption, no consistent relationships between total oxygen consumption and skin temperature or peripheral blood flow were demonstrated.

**The effect of acetyl-beta-methyl-choline on blood flow through the hand at low temperatures** MARTIN B. MACHT, MORTIMER E. BADER and ELIZABETH L. PILION (introduced by H. S. BELDING) *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Massachusetts*. Utilizing a 0.2% solution of acetyl-beta-methyl-choline as the conducting medium in a metal venous occlusion plethysmograph, this drug was passed through the skin of the hand by iontophoresis. With the plethysmograph serving as the positive electrode and a saline saturated negative electrode placed on the subject's back, a current of 10 milliamperes, derived from a 45 volt B battery through a 10,000 ohm variable resistance, was used to effect ionization. A thermostatically regulated refrigerating coil and heating unit within the plethysmograph permitted accurate control of hand skin temperature at any desired level throughout the experiment. All studies were conducted in a constant temperature room.

At a room temperature of 15°C and a plethysmograph temperature of 10°C, blood flow was drastically curtailed in the hands (less than 0.8 cc/100 cc limb volume/minute). Iontophoresis of ABMC un-

der these conditions resulted in a marked increase in blood flow of the treated hand in three of the four subjects studied. Maximum flows amounting to as much as twenty times the control flow were observed. The augmentation in flow usually occurred within fifteen minutes after the iontophoresis began, reaching a maximum within thirty minutes. Systemic effects were negligible or absent, and no changes in skin temperature or blood flow of the untreated hand were noted. No effect resulted from ABMC without current, or from iontophoresis of normal saline. The ability of ABMC to overcome locally the vasoconstriction produced by cold may be of major significance in studies of peripheral vascular adjustments to environmental temperature and warrants further investigation.

**Responses to small changes of light intensity by the light-adapted photoreceptor** E. F. MACNICHOL (by invitation) and H. K. HARTLINE *Johnson Research Foundation, University of Pennsylvania, Philadelphia*. Action potentials were recorded from single optic nerve fibers from the eye of *Limulus*. The preparation was subjected to a constant illumination lasting for several seconds. When the discharge of nerve impulses reached a constant frequency the illumination was changed by small increments and the time course of the change in the frequency of discharge was followed. In response to a small increase in illumination a large transient increase in frequency was produced. The frequency eventually subsided to a steady level slightly greater than that preceding the change in illumination. A small decrease in illumination was likewise followed by a large decrease in frequency followed by a gradual recovery to a level slightly below that previous to the change in illumination. If a flash of light lasting several seconds was added to a steady background of illumination the transient at the beginning and end were nearly but not exactly symmetrical, the number of impulses added at the onset of the flash was very nearly equal to the number of impulses missed at its termination. The magnitude of the transient change in frequency depended on the level of background intensity and on the magnitude of its increment, and was approximately constant for a fixed incremental ratio. Duration of the intensity increment and of the foregoing period of steady background illumination affected the magnitude of the transient.

**Antigonadotrophin formation to sheep FSH effectiveness against endogenous gonadotrophic hormones in men** WILLIAM O. MADDOCK, EDWIN C. JUNGCK (by invitation), and CARL G. HELIER *Department of Physiology, University of Oregon Medical School, Portland, Oregon*. Seven infertile men, each having some functional testicular tissue and detectable amounts of urinary gonadotrophins (with one exception) were given intramuscularly 50 to 100 rat units of a purified follicle-stimulating

indicating at least two components in the prothrombin conversion factor. The concentration of prothrombin conversion factor in or on the platelets appears to be related to the function of platelets as foci in coagulation of the blood.

**The separate contraction of functional areas in limb muscles during reflex withdrawal and crossed extension.** J. E. MARKE and MAUD WILLIAMS (by invitation) *Dept. of Anatomy, Duke University School of Medicine, Durham, North Carolina and Division of Physiology, Women's College of U. N. C., Greensboro, North Carolina.* Previously we have demonstrated in the dog that the separately innervated areas within a muscle may contract separately by electrical stimulation of their nerves, and in the human, that the arrangement of the nerve branches to the long limb muscles is similar. In this study we recorded, photographically and electrically, the shortening of separately innervated areas under reflex conditions—ipsilateral withdrawal and crossed extension—of all the limb muscles which move both hip and knee joints. Sixty-five decerebrate and spinal dogs were used.

The predominate reflex response of the long thigh muscles is as follows. In the semitendinosus the proximal part shortens during extension of the hip, the distal part, during flexion of the knee. The semimembranosus shortens during flexion of the knee only. The lateral part of the biceps femoris shortens during knee flexion, the medial, during hip extension. The caudal part of the sartorius shortens only during knee flexion, the cephalic cranial part during hip flexion, the distal cranial part during knee extension. The superficial part of the rectus femoris shortens during knee extension, the deeper part, during hip flexion. The superior part of the gracilis shortens during knee flexion, the inferior part, during hip extension. Certain different functional areas of a muscle, under reflex stimulation, react as if reciprocally innervated. Action potentials were recorded in areas that shorten, but not in areas that lengthen.

**Purification of renin.** JOHN MARSHALL (by invitation) and G. E. WAKERLIN *Department of Physiology, University of Illinois College of Medicine, Chicago.* A modification of earlier methods for the purification of renin was developed for the purpose of producing large amounts of relatively pure hog renin in high yields. Dried hog kidney powder prepared by acetone ether extraction was used in lots of 10 kg. of fresh kidney equivalent. Each lot was extracted with saline-bicarbonate at a pH of 8. The extract was filtered through muslin, and inert protein precipitated at pH 2.90 at an NaCl concentration of 0.92 M, using HCl (2N) to pH 4 and trichloroacetic acid (10%) to pH 2.90. After centrifugation the supernatant was saturated with NaCl and the precipitate of crude renin was separated by

centrifugation. This was then dialyzed until essentially salt free. Total volume was 1 liter, containing 16 Goldblatt renin units per ml., at a purity of 8 units per mg. N. Further purification was achieved by ethanol fractionation under controlled conditions of ethanol concentration, pH, ionic strength, temperature, and protein concentration. Inert protein was removed at pH 7.0, EtOH 17%,  $\gamma/20.01$  to 0.603,  $T = -5^\circ$ . This precipitate consisted of 10–15% of the total protein and 10% of the total renin. (Increasing ionic strength or pH yielded less protein, and increasing ethanol to 20% precipitated 60% of the renin.) After centrifugation at  $-5^\circ$ , the supernatant was adjusted to pH 4.90 with 0.3 M acetate buffer. The precipitate formed contained about 15% of the initial total protein, and 80–90% of the total renin at a purity of 50 units per mg. N. Further purification with minimal loss of renin was achieved by a second fractionation at lower ionic strength and ethanol concentrations. This work is continuing.

**The rate of atrophy of rat diaphragm.** ARTHUR W. MARTIN and O. M. SOJA (by invitation) *Department of Physiology and Biophysics, University of Washington, Seattle, Washington.* The rate of muscle atrophy varies approximately inversely with increasing animal size. While it has not been possible to relate the rate of atrophy directly to the rate of oxygen consumption, Diaz Guerrero and Thomson (*Fed. Proc.* 6:97, 1947) showed that thyroxine administration increased and thiouracil decreased the rate of atrophy of muscle. It appeared of interest to compare the rate of atrophy of a muscle with a high metabolic rate with one of low metabolic rate in the same species of animal. Field, et al. (*J. Cell Comp. Physiol.* 14, 113, 1939) showed that rat diaphragm consumed oxygen at about twice the rate of other skeletal muscles. Abundant data have been obtained on the rate of atrophy of other muscles of the white rat, particularly following denervation. We have therefore determined the rate of atrophy of a hemi diaphragm in this species following section of the left phrenic nerve in the thorax. Seven days after nerve section a maximum hypertrophy of 50 per cent had taken place, although by this time gastrocnemius muscle would have atrophied about 23 per cent. The hypertrophy was gone after 30 days by which time gastrocnemius atrophy would be about completed (75 per cent). Progressive atrophy followed, a 25 per cent weight loss occurred by 70 days, a 50 per cent loss by 120 days.

No significant changes in absolute amount of connective tissue took place within 120 days. Even when allowance was made for the amount of connective tissue the  $QO_2$  was significantly elevated (22 per cent) during hypertrophy and reduced during atrophy.

**The effects of various pressure application de-**



hormone prepared from sheep anterior pituitaries (Schering's FSH, containing small amounts of ICSH and thyrotrophin) daily for 60 to 105 days. Anti gonadotrophins, although not demonstrable before treatment, appeared in the plasma from 49 to 60 days after beginning therapy. These titers were sufficiently great to nullify the effect of 100 times the amount of FSH administered. Highest titers were reached at the time of stopping therapy. At this time 0.9 cc of plasma completely inhibited the effect of 2 rat units of FSH administered to immature rats (usually 2 rat units of FSH caused an increase in ovarian weights from circa 13 to circa 30 mgm). Four to five months after cessation of therapy anti gonadotrophin titers were still approximately half as high as peak levels. Injecting FSH did not cause a rise in urinary gonadotrophin output. Detectable amounts of urinary gonadotrophin were found at the time of maximal anti gonadotrophin formation.

The patient's plasma anti gonadotrophin capacity was tested against the patient's own urinary gonadotrophins in several instances, and also against other patients' urinary gonadotrophins, and were effective against both. Therefore, either the patients' kidneys or the ultrafiltration concentration method separated endogenous gonadotrophin from endogenous anti gonadotrophin. This indicates that anti gonadotrophins do not destroy, inactivate or irreversibly combine with gonadotrophins.

**Effect of hydrogen and bicarbonate ions on the metabolic rate of the perfused cat brain.** J. MAGNES and A. GEIGER (introduced by H. GRUNDFEST) *Department of Physiology, the Hebrew University, Jerusalem and Department of Psychiatry, College of Physicians and Surgeons, Columbia University.* Isolation of the cerebral circulation and perfusion of the brain in the living cat, by the method described previously (A. Geiger and J. Magnes, *Amer Journ of Physiol* 149: 517, (1947)), offers an opportunity to study the effect of blood composition on the metabolic functions of the brain. The effect of  $(H^+)$  and of  $HCO_3^-$  was studied while perfusing the brain with defibrinated heparinized ox blood. Acidifying the blood with HCl or with lactic acid increased cerebral oxygen consumption considerably, for instance, a change from pH 7.25 to pH 7.20 usually caused an increase of 25-30% in oxygen consumption. Addition of  $NaHCO_3$  caused a corresponding depression. On the other hand comparable changes in hydrogen ion concentrations, brought about by varying the  $CO_2$  tension of the perfusing blood were inconsistent in their effect on brain metabolism and were often without any influence whatsoever. The metabolic rate of the brain is highest when perfused with blood having a low  $NaHCO_3$  concentration. (This was obtained by dialysing blood against 0.9% NaCl, and subsequently adding all the electrolytes and

glucose in physiological concentrations with the exception of bicarbonate, which was kept low.) These experiments suggest that hydrogen ions stimulate, and bicarbonate ions depress, brain metabolism.

**Urea-clearance by perfusion of the entire intact small intestine in man.** N. S. R. MALUF *Department of Pharmacology, University of Louisville School of Medicine, Louisville, Ky, and the Louisville General Hospital.* Perfusion was through a modified Miller-Abbott tube. The 300 cm.-long, 16 French modified tube has three conduits: 1 for inflating the terminal balloon when the tube has entered the duodenum, 2 for passing the perfusing liquid into the proximal jejunum through 5 holes about 150 cm (5 ft) from the distal end, 3 for sucking back the liquid at the distal ileum through 5 holes immediately proximal to the balloon. The balloon is deflated when the distal end of the tube is in the lower ileum. The solution was somewhat hypertonic  $Na_2SO_4$ . Suction was set to read -5 to -10 mm Hg at junction of intestinal tube and tubing connected to reservoir and suction-pump. With the rate of perfusion at 30 cc/min, the concentration of urea in the outflow liquid was almost identical with that in the plasma, giving a urea-loss of 11.6 Gm in 8 hours, or a urea clearance of 32.4 cc/min. This compares well with peritoneal irrigation and external dialysis ("artificial kidney"). The method is much less cumbersome and requires no asepsis or surgery; furthermore, it may be temporarily discontinued when desired. The method is indicated in temporary acute renal insufficiency, as after transfusions with incompatible blood, sulfonamides, or in crush-syndrome. The chloride loss in 8 hours was 6.64 Gm (as NaCl), this can be readily replaced by NaCl by intravenous drip. As shown by Amberson,  $SO_4$  may replace Cl to a large extent without observable injury.

**The platelets as foci in the coagulation of the blood.** F. D. MANN, MARGARET HURN and D. R. MATHIESON (introduced by T. B. MAGATH) *Division of Clinical Laboratories, Mayo Clinic, Rochester, Minnesota.* The coagulation of normal human plasma in silicone coated slide preparations was observed by phase contrast microscopy. As soon as fibrin threads were visible they were seen radiating from the platelets. Thus the platelets appeared to serve as foci of fibrin formation, but simultaneous formation of fibrin free in the plasma is not excluded. After coagulation the platelets were firmly and for the most part individually fixed in the fibrin network and did not migrate. The relative prothrombin converting activities of plasma and of platelets were studied with the aid of aged, zymotreated and ammonia treated plasma. The ability to restore the coagulability of aged and ammonia-treated plasma was greatly concentrated in or on the platelets. This agrees with previous evidence

indicating at least two components in the prothrombin conversion factor. The concentration of prothrombin conversion factor in or on the platelets appears to be related to the function of platelets as foci in coagulation of the blood.

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**The effects of various pressure**

414, 1947) showed that certain sugars present in gastric mucin and pectin can increase the utilization of alpha tocopherol in progressive muscular dystrophy. Alpha tocopherol alone was without effect on creatinuria, when given together with D-Mannose, D-Galactose or L-Fucose alpha-tocopherol induced pronounced reduction in the creatine output of suitable subjects. Through the kindness of Dr. James G. Baxter of Distillation Products, Inc., Rochester, N. Y. who supplied us with samples, we have had the opportunity of testing the effects of gamma tocopherol and delta-tocopherol. The patients who were given test doses of these substances were maintained on a constant creatine free diet and had been used in the earlier investigations of alpha tocopherol. Gamma-tocopherol administered in daily doses of 330 mg for 3 days either alone or with D-Mannose or raffinose was without effect on creatinuria. On the other hand, delta-tocopherol given for similar test periods in amounts of 220 mg daily reduced the creatinuria as much as 40 per cent for 9 days in some patients. The effect was greatest in patients with benign forms of the facial-humeral scapular type of muscular type of muscular dystrophy, and less, or even absent, under these conditions, in patients with rapidly progressive forms of the pseudo hypertrophic type of the disease.

**The influence of oxygen administration on the cardiovascular response to exercise.** A. T. MILLER, Jr., *Laboratory of Applied Physiology, University of North Carolina, Chapel Hill.* Three subjects, thoroughly trained in treadmill running, performed standard exercise which consisted in running on the treadmill (speed 7 m p h, grade 17.5 per cent) for 5 minutes. Heart rate and systolic blood pressure were recorded at one minute intervals during exercise and 20 minutes recovery. Blood lactate concentration was determined before and after exercise and after 20 and 60 minutes recovery. Oxygen was administered for 10 minutes before exercise, during exercise, during a 20 minute recovery period and during a 5 minute interval between two identical exercise periods in different series. Each series consisted of 3 or more experiments on each of the 3 subjects, with an equal number of control experiments in which air was breathed.

Two of the subjects made repeated exhaustion runs (3 minutes at 12 m p h, 17.5 per cent grade) in addition to the standard runs. The results may be summarized as follows: (1) Oxygen administration *before* exercise had no effect on heart rate, blood pressure and blood lactate during exercise and recovery. (2) Oxygen administration *during* exercise had no effect on heart rate or blood pressure during exercise or recovery. Maximal blood lactate concentration was reduced to a slight but statistically significant extent (confirmed by ex-

periments on 20 additional subjects). (3) Oxygen administration *during recovery* had no effect on the rate at which heart rate, blood pressure and blood lactate returned to normal. (4) Oxygen administration between double runs had no effect on heart rate, blood pressure and blood lactate during the second run and recovery.

**Insulin excretion in normal man.** I. ARTHUR MIRSKEY, JOHN WACHMAN (by invitation), C. J. PODORE (by invitation) and R. H. BROTH-KAHN (by invitation). *May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio.* The amounts of insulin excreted into the urine by normal subjects were determined by the following procedure. Several days' urine from each subject was desiccated by lyophilization. The resultant powder was then extracted by the usual acid alcohol procedure and the insulin recovered by precipitation with ether. The insulin content of the final acid saline extract was determined by the Young modification of the mouse dropping test. Recovery experiments indicated that from 60 to 100 per cent of 0.5 unit of insulin added to large volumes of urine could be detected by this procedure. Numerous trials have indicated that under ordinary conditions of diet, normal subjects excrete very small amounts of insulin into the urine. The average amount of insulin determined in a series of such normal subjects was found to be about 0.3 units per day. Further trials have indicated that, even after the injection of fairly large amounts of insulin into normal subjects, little additional insulin appeared in the urine. This suggests that normal man excretes only minute amounts of insulin into the urine. In view of the probability that exogenous insulin rapidly disappears from the blood stream of man, these findings would be indicative of the very rapid rate of destruction of insulin by the intact organism.

**Calibration of the Millikan compensated oximeter as used among white and colored persons.** GEORGE E. MONTGOMERY, JR. (by invitation), J. E. GERACI (by invitation) and EARL H. WOOD. *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota.* The Millikan CMR model no. 13 compensated circuit oximeter has been calibrated against Van Slyke analyses of simultaneously drawn samples of arterial blood from twenty-five normal white persons and five normal Negro persons. Multiple samples of arterial blood were obtained by the technique described by Wood and associates (see abstract). Hypoxia was produced by allowing the subjects to breathe gas mixtures of from 16 to 7 per cent oxygen content. Ten different Coleman type earpieces were used. Five hundred fourteen simultaneous oximeter and Van Slyke determinations of arterial oxygen saturation were made in the group of twenty-five white persons. In the saturation range from 50 to 91 per cent, the

the lower thoracic cord and upper three lumbar segments, presumably Clarke's column, where is Group II fibers, of muscle and cutaneous origin, contribute to the tract of Goll

**On estimating the relative volumes of corpuscles and serum in blood** PAUL L. McLAIR and C. H. WILLIAM RUIHR (introduced by C. C. GURRINI) *Department of Physiology and Pharmacology, School of Medicine, University of Pittsburgh* Relative corpuscle and serum volumes were estimated in numerous samples of defibrinated beef blood by 12 technically different applications of the serum dilution principle. The results were compared with those obtained on the same samples by centrifugation at approximately 7500  $\times$  G. In the dilution studies, the following measurements on serum were used as indices: specific gravity by copper sulfate and falling drop methods, total nitrogen, serum protein estimated from nitrogen determination and from specific gravity, heat coagulable serum solids by direct weighing and from specific gravity, concentration of the dye T-1824. With regard to relative corpuscle volume, the means for all dilution methods were smaller than that for the conventional hematocrit, differences varying with the method from 1 to 20 per cent of the mean cell volume. The average differences, for comparisons on individual samples, between centrifugal and dilution procedures covered a similar range. In general, the dilution methods which give the smallest deviations from the centrifugal, especially those which involved washing the serum from the packed corpuscles, were the most consistent. However, present results do not justify the selection of any one method as estimating reliably the "true" cell volume, or the practice of correcting conventional hematocrit results by the application of any constant factor based on dilution methods.

**Preliminary study of complete parenteral alimentation** H. C. MENG (by invitation), and SMITH FREEMAN *Department of Physiology and Department of Experimental Medicine, School of Medicine, Northwestern University, Chicago, Illinois* Two adult male dogs were fed a fat free synthetic diet by mouth plus 10% butter oil emulsion by vein for 4 weeks, after which the animals were infused exclusively through the intravenous route with mixtures containing all the nutritional elements in adequate amounts except water which was allowed by mouth *ad libitum*. Two types of mixture were used. One contained 10% butter oil in emulsion with the stabilizers—span 20, "Asolecten" and sodium cholate—and 10% glucose. The other contained a 10% casein hydrolysate ("Amigen"), glucose, salt mixture and water soluble vitamins. Fat soluble vitamins and liver extract were injected intramuscularly every week. The total daily caloric intake was 92.65 calories per kilogram of which 49% came from carbohydrate, 19% from protein and 32% from fat.

On some occasions the butter oil emulsion was omitted and the calories from the fat were made up by glucose. The animals were infused for 8 and 10 weeks. There was no change in total plasma proteins, plasma N P N, Rose Bengal clearance and serum phosphatase. Their body weight was apparently maintained. The nitrogen balance was positive except on 1 or 2 occasions. No pyrogenic effects were observed. Vomiting occurred when infusion was given too rapidly. A moderate reduction of hemoglobin, red cell count and hematocrit was noticed after the first week of injection, but did not progress appreciably thereafter. Hematuria occurred on several occasions. Further studies are in progress.

**Removal of rostral border of human area 4 followed by spasticity or lack of it** FRED A. METTLER and J. LAWRENCE POOL (by invitation) *Department of Neurology, College of Physicians and Surgeons, Columbia University* Hines and Tower showed that removal of the caudal part of the simian area gigantopyramidalis produced no spasticity and imputed such spasticity as does follow removal of area 4 to damage of its rostral border. Mettler (1943, *J. comp. Neurol.* 79: 185-215) demonstrated that unilateral decortication produces negligible spasticity but that progressively deeper ablations ultimately produce the complete hemiplegic picture. Four human cases are here presented. In case K, area 6 (except caudal border) and rostrally adjoining cortex was unilaterally removed. Stimulation still gave good movements. Postoperative paralysis with resistance to passive movement developed. In case M the caudal third of the middle frontal gyrus was unilaterally removed without result. The precentral and part of the postcentral gyrus caudal to the original ablation were next removed. A flaccid paralysis developed from which the patient recovered without showing evidence of spasticity. In cases E and Y, area 6 was bilaterally removed without eliciting any evidence of spasticity. Subsequently the rostral part of area 4 was removed unilaterally in both cases. In case Y a pronounced spastic hemiplegia developed, in Case E a flaccid paralysis ensued. Since cases K and Y had pre-existing subcortical pathology and cases M and E did not, it is concluded that unilateral removal of neither area 6, nor of 6 and the rostral part of 4, nor of 6 and both the rostral and caudal parts of 4 (and the rostral part of the postcentral gyrus) produces notable spasticity in the human unless subcortical damage also exists.

**Effect of delta- and gamma-tocopherol on creatinuria in progressive muscular dystrophy** A. T. MILHORAT (*Depts. of Psychiatry and Medicine, Cornell Univ. Medical College, The Russell Sage Institute of Pathology and the New York Hospital, New York, N. Y.*) Investigations reported previously (Milhorat and Bartels, *Federation Proc.* 6

the nervous system have been employed in an analysis of its role in the maintenance of the blood pressure level in experimental renal hypertension. The results bear out the following conclusions. The applications of various drugs which produce surgical anesthesia in dogs demonstrates by the changes in pressure levels that there is no quantitative difference in the role of the nervous system in the maintenance of the blood pressure level in normotensive, early renal hypertensive, or late renal hypertensive dogs. There is a large increase in the role of the nervous system in the maintenance of the pressure level in neurogenic hypertensive dogs. Blocking the visomotor outflow between the central nervous system and the blood vessels causes changes in the blood pressures of dogs which also demonstrate no increased role for the nervous system in the maintenance of the blood pressure level in renal hypertensive dogs in either early or late stages. *Neurogenic hypertension is again shown to involve an increased role of the nervous system.* Studies of reflex mechanisms in normotension and late renal hypertension show little difference in the responses to hypercapnia and cold stimulation. The responses of early renal hypertensives to these stimuli are somewhat less. Insofar as these experiments are concerned, the role of the nervous system in the maintenance of the blood pressure level in experimental renal hypertensive dogs does not appear to differ from its role in normotensive dogs.

A polarographic study of the action of carbonic anhydrase at the dropping mercury electrode. OTTO H. MÜLLER, *Dept of Physiology, Syracuse University College of Medicine, Syracuse, N. Y.* In a polarographic study of quinydron in buffered and unbuffered solutions it was found that the carbon dioxide bicarbonate buffer behaved abnormally (Müller, *J. Am. Chem. Soc.*, 1940, 62, 2131). Instead of a single wave in which the anodic and cathodic portions overlap, two waves were obtained which were similar yet not identical with those found in an unbuffered solution. This was attributed to a slowness in the response of the buffer to changes in pH at the electrode surface. If carbonic anhydrase (prepared from sheep erythrocytes by the customary alcohol chloroform method) is present in sufficient concentration and all inhibitors (such as  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , etc.) are absent, the carbon dioxide bicarbonate buffer reacts with sufficient rapidity to maintain constant the pH at the dropping mercury electrode during the reduction or oxidation of quinydron. This is evident from the fact that the anodic and cathodic waves now overlap completely and form a single wave, to separate again more or less as the enzyme is destroyed or inhibited by well known inhibitors.

**Composition of Central American foods. I. Honduras.** HAZEL L. MUMFORD, ROBERT S. HARRIS and LOUIS O. WHITMAN (by invitation), with the tech-

nical assistance of LOUISE GUILD (by invitation), GERTRUDE NICHINGALL (by invitation) and CHARLES THOLSCHER (by invitation). *Nutritional Biochemistry Laboratories, Massachusetts Institute of Technology.* For the establishment of a sound program of food production and food consumption it is important to know the composition of the indigenous foods which are, or can be, grown in the area. On this basis a long range study of the composition of foods grown in Central America is underway. Specimens are collected and identified by a botanist, samples representative of the edible portions are stabilized and shipped by air express to Cambridge. Whenever possible a record is made of the soil type, annual rainfall, altitude and whether the sample was grown on fertilized land. A comparison of the data from similar samples may give information on the relative importance of genetic and non genetic factors in determining the composition of edible plants. *Moisture, nitrogen, ether soluble fraction, crude fiber, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin and total ascorbic acid content* are being measured. In the present report the results of analyses of 129 samples of 85 plants collected in Honduras are presented.

**The effects of the intravenous administration of glucose and amino acids on the hepatic blood flow and splanchnic oxygen consumption of man.** By J. D. MILLS (Introduced by E. A. STAD, JR.), *Dept. of Medicine, Duke University, School of Medicine, Durham, N. C.* The work of Wilhelmj, Bollman and Mann on the dog, and of Dock on the rat, has indicated that the specific dynamic action of proteins and amino acids takes place chiefly, if not solely, in the liver. Munn and Boothby have established that in the dog the specific dynamic action of glucose is not only still present after hepatectomy but is actually accentuated in the absence of the liver. The estimation of hepatic blood flow by bromosulphalein clearance during catheterization of the hepatic veins, and the calculation of the splanchnic oxygen consumption (the estimated liver blood flow  $\times$  the hepatic arterio-venous oxygen difference) have provided means of investigating the immediate effects of the intravenous administration of amino acids and glucose on the hepatic blood flow and splanchnic oxygen consumption in a group of hospital patients without significant disease. The rapid intravenous injection of 50 grams of a mixture of amino acids in 10 per cent solution (Vujn 9 solution—Merek) has resulted during the subsequent hour in an increase in splanchnic oxygen consumption to as much as 250 per cent. This is accomplished by a marked increase in hepatic arterio-venous oxygen difference with no significant change in hepatic blood flow. At the same time, there is an outpouring of glucose from the liver as evidenced by an increased hepatic venous arterial glucose difference. The similar in-

average difference between the oximeter and the Van Slyke values was +1.7 percentage points (range, -1.1 to +2.3 percentage points). The accuracy of the oximeter was found to vary significantly when different capillaries were used. The average differences between photoelectrically and chemically determined arterial oxygen saturation values ranged from +1.4 to +13.1 percentage points for the individual capillaries tested. Sixty-two comparisons of oximeter-determined and Van Slyke determined values of the arterial oxygen saturation were made in a group of five Negro and five white persons, the same oximeter capillary being used. In the 50 to 90 per cent saturation range in Negroes, the average difference between Van Slyke and oximeter values was +12.8 (1.7 to 22.7) percentage points. In the white persons this value was +7.1 (-1.1 to 15.7) percentage points. Figures in parentheses are extreme values.

**Estimation of the total circulating red cell volume by the use of methemoglobin-tagged cells.** JAMES C. MOORE and O. W. SHADLE (introduced by HAMPDEN C. LAWSON) *The Dept. of Physiology, Univ. of Louisville School of Medicine.* Sodium nitrite was added to heparinized blood in the proportion 50 mgm./100 cc. for *in vitro* methemoglobin formation. The methemoglobin containing cells were washed with saline and injected intravenously as concentrated cell suspensions in volumes of 35-70 cc. in barbitalized, splenectomized dogs. Methemoglobin was determined spectrophotometrically by the method of Horecker and Brackett on arterial samples drawn at intervals of one to five minutes after the injection. Methemoglobin disappearance curves similar to those found *in vitro* were obtained after the first five minutes. Arterial methemoglobin at the instant of injection was obtained by extrapolation of these curves. Cell volume was calculated from the total methemoglobin injected and the packed-cell methemoglobin concentration in arterial blood, both values being read at the instant of injection, the latter by extrapolation as above. *In vitro*, this method gave values for cell volume within 2 per cent of those obtained from the hematocrit and the total volume.

In a series of six dogs comparative values for cell volume were obtained by the present method, by calculation from the hematocrit and the plasma volume, and by the "hematocrit-change" method of Lawson, Overbey, Shadle, and Moore. The second method gave values ranging from 126 to 152 per cent, and the third method 91 to 130 per cent of the volumes obtained by the present method.

**Studies of shock in frogs.** 1. **Shock due to electrical injury.** LOUIS MOREAU (by invitation), MARVIN BALISTOCKY (by invitation), and L. V. HILBRUNN *Zoological Laboratory, University of Pennsylvania.* The frog is an excellent animal in which to study shock phenomena. Some of the com-

plications which enter into the shock picture in mammals are not present in the frog. We have found that shock may be caused by electrical injury to a restricted region of the animal. If either the brain or the legs are subjected to currents sufficiently strong to cause injury (but not excessive heat) the animals show obvious shock. Toxic factor is involved, for ligation of the legs prevents shock in frogs whose legs are exposed to electrical injury. The toxic factor is apparently of the nature of a thrombin or thromboplastin. This is indicated by the changes which occur in the coagulation time of the blood of shocked animals. The first change is a slight decrease in the coagulation time and this is followed by an increase.

**The effect of intraarterial injection of acetylcholine upon the gastric mucosa of the dog.** G. M. MORTON (by invitation) and G. W. STAVRAKY *Departments of Anatomy and Physiology, Faculty of Medicine, University of Western Ontario, London, Canada.* As reported by STAVRAKY (*Federation Proc.* 14, 1915) acetylcholine, when injected into the gastrosplenic artery, causes a secretion of alkaline gastric juice. A histologic study of the gastric mucosa of the injected region of the stomach—a limited area of the body along the greater curvature—showed that after 4-6 hours of injection of acetylcholine, complete exhaustion of the chief cells of the neck of the gastric glands and partial exhaustion of the surface epithelium took place. In contrast to the quiescent areas of the stomach, when stained with mucicarmine, the chief cells of the neck of the glands contained no secretory granules, their cytoplasm appearing quite clear. The cells of the surface epithelium, particularly those in the depths of the gastric pits, contained diminished amounts of the mucus precursor, the secretory granules occupying only the outer regions of the cells and leaving a clear area between the mass of the granules and the nucleus. When stained with neutral gentian, the chief cells of the body of the glands were found to contain large numbers of pepsinogen granules showing no difference between the injected and resting mucosa. This latter was in keeping with the absence of any digestive power in the secreted juice.

It is concluded that the highly alkaline gastric juice (pH up to 8.9) which is secreted in response to injections of acetylcholine into the gastrosplenic artery comes from the inner one-third of the glands of the body of the stomach along the greater curvature, the chief cells of the neck and possibly the surface epithelium being responsible for its secretion.

**The role of the nervous system in experimental hypertension in the dog.** W. G. MOSS (by invitation) and G. E. WAKERLIN *Department of Physiology, University of Illinois College of Medicine.* A variety of techniques for the study of the role of

the adult animals seemed to be in good condition until one or two weeks before their death, at which time they lost weight and a few developed tremors and ataxia. None showed any evidence of edema.

The effect of diet on liver regeneration in partially hepatectomized rats. EDWARD A. NEWMAN (by invitation) and MORTON I. GROSSMAN, *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago*. Based on the recent works of Denton and Ivy, studies were made to determine whether the enhancement of liver regeneration following partial hepatectomy by feeding liver is due to the high protein content of liver. Four series of experiments were run in the following manner. Partially hepatectomized rats were fed: Group A, 60% casein diet; Group B, coagulated beef heart; Group C, 60% powdered liver diet; Group D, coagulated liver. The rate of regeneration was estimated by sacrificing all the animals eleven days after surgery and calculating the ratio of dried regenerated liver weight to the body weight. Rats fed coagulated hog liver showed a ratio of 0.0115. The next greatest rate of regeneration was seen in rats given 60% powdered liver diet with a ratio of 0.0104. The remaining groups showed a poor regenerative response as seen by the respective order of ratios: coagulated beef heart diet, 0.0078, and 60% casein diet, 0.0082. The average body weight of the rats in all groups was within 5 grams of 300 grams. These results indicate that the enhancing effect of liver is not due to its high protein content.

Electrocardiographic changes on tilting. MICHAEL NEWTON (introduced by H. C. BAZETT), *Department of Physiology, Medical School, University of Pennsylvania, Philadelphia*. Important circulatory adjustments occur on changing from the horizontal to the vertical position. Horwitz (personal communication) noted that a group of patients with possible angina pectoris showed a relative lengthening of electrical systole on tilting to the vertical position and a relative shortening of systole on tilting back to the horizontal. The time relations of these changes were investigated electrocardiographically during studies on the effect of change of environment and other procedures in healthy young men. From Bazett's formula ( $\text{systole} = K\sqrt{\text{cycle}}$ ) if  $K$  is high, systole is long relative to the cycle, while if  $K$  is low, systole is short relative to the cycle. On tilting from the horizontal position to 70° from the horizontal the value of  $K$  rose to a maximum within 60 seconds, it then decreased to a level which was somewhat above the resting value during the twenty minutes of observation. On tilting back to the horizontal the value of  $K$  was greatly decreased, over a five minute period it gradually returned to the resting level. The factors involved in these changes are complex and not easily explained. They are probably chiefly mechanical (venous return) and nervous (vagus and sympathetic influences). The changes observed in the

value of  $K$  were not significantly affected by changes in environmental temperature, by hemorrhage or by infusion of serum albumin.

Blood volume determination by plasma dye dilution and dilution of red cells tagged with P 32. ROBERT T. NIESER (introduced by H. S. MAYERSON), *School of Medicine, Tulane University and the Research Division of the Alton Ochsner Medical Foundation*. Independent studies in patients on the rate of absorption and of loss of radioactive phosphorus by red cells in vivo and in vitro and of the loss of phosphorus from the plasma in vivo have been made to check the validity of red cell volume determinations by a simple dilution method using radioactive phosphorus as a tracer. Whole blood samples are used for counting so that no chemical or physical separation of the trace element is required. Discrepancies between whole blood volume calculated from the red cell volumes and that calculated from the plasma volumes as determined by plasma dye (T1824) are analyzed with reference to the ratio of plasma volume to red cell volume. Variations in plasma to cell volume ratios are shown to have opposite effects on the apparent volumes so that the results are not directly comparable. Total blood hematocrits are obtained from the independent measurements of total plasma volume and total red cell volume and are compared with direct hematocrit readings made from peripheral blood. The variation appears to occur in either direction and to be non-specific.

Effect of iodine in hypothyroid rats. WARREN O. NELSON and HELEN E. WHEELER (by invitation), *Department of Anatomy, University of Iowa College of Medicine*. Female rats made hypothyroid by thyroidectomy or administration of thiouracil or thiourea show disturbances of reproductive rhythm, small ovaries and adrenals, and retarded growth. Periods of estrus are very irregular and frequently may be absent for several weeks. When they do occur they are accompanied, as a rule, by ovulation of a few follicles only. It has been observed that the administration of  $I_2$ , 6 mg and  $KI$ , 6 mg in one-tenth cc water daily will prevent or correct these various departures from normal. A partial effect only was achieved in animals which received 3 mg  $I_2$  and 6 mg  $KI$  daily. The procedure employed in routinely evaluating the effect of iodine upon hypothyroidism produced by various drugs or by thyroidectomy has involved observation of groups of 12 to 16 animals for 30 days. This serves to establish adequately the hypothyroid state. During the following 30 days one-half of each group receive, in addition, iodine by subcutaneous injection. Estrous cycles usually show improvement within ten days and normal reproductive rhythm is re-established. Ovaries and adrenals are approximately doubled in size and livers, kidneys and spleen show significant weight gains. Iodine treated animals average approximately 30 grams heavier and 2 cm

longer at the close of the 30 day period than animals not receiving iodine. The thyroids of rats treated with thiourea or thiouracil plus iodine are smaller than those in rats not receiving iodine. It is supposed that the effects noted are achieved through extra-thyroidal iodination of protein.

**Heart temperature and its relation to the T-wave.** L F NIMS, B KARTIN (by invitation), I M CHERNOFF (by invitation) and L H NAHUM *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn.* The temperature of the dog's heart was determined by means of thermocouples inserted at several points in the pericardium. The heart temperature averaged about 0.25°C above rectal temperature, except at the diaphragmatic surface which was about 0.50°C above rectal temperature. An open chest dropped heart temperature and altered the T waves in a characteristic manner. On closing the chest, the temperature gradually rose and stabilized after 90 minutes. A pint of hot or cold water placed in the stomach maximally affected the temperature of the diaphragmatic surface. Heat or cold transfer through the anterior chest wall by means of radiant bulb or ice pack maximally altered anterior heart temperature. Various areas of the heart surface were heated and cooled directly by means of a chamber inserted in the pericardial sac in an attempt to quantitate T-wave change in standard and unipolar leads against temperature change. For every 1°C change in temperature of the left ventricle a 1 mm change in amplitude of the T-wave was observed, while similar change in temperature of the right ventricle resulted in an average 0.5 mm change in T-wave amplitude, but in the opposite direction.

These experiments suggest that normally occurring temperature differences in various parts of the heart are of sufficient magnitude to account at least in part for the amplitude and direction of the T-wave.

**The effect of carbon dioxide on the motility of the small intestine.** DAVID W NORTHUP, J C STICKNEY, and E J VAN LIERE *West Virginia University Medical School, Morgantown, West Virginia.* Previous reports from this laboratory have shown that anoxia alone has no effect on the motility of the small intestine of the dog (Am Journ Physiol 140:119, 1943). In asphyxia, carbon dioxide accumulates in the tissues, therefore experiments were performed in which 6.5–8.0% CO<sub>2</sub> was added to the inspired air to determine the effect of this factor alone—i.e. no anoxia and no asphyxia. In dogs breathing this concentration of CO<sub>2</sub> a charcoal gum acacia mixture administered by stomach tube traversed an average of 110 cm of small intestine in 30 min, in the controls, an average of 165 cm. This reduction in motility is significant at the 4% level.

**Influence of anti-organ sera upon the oxygen**

**uptake of the spleen and brain.** WIKTOR W NO WINSKI (introduced by C M POMERAT) *Tissue Culture Laboratory and the Psychopathic Hospital, Medical Branch, The University of Texas, Galveston, Texas.* The fact that Reticulo Endothelial Immune Serum (REIS) in strong concentrations has an inhibitory effect upon the migration of cells in tissue cultures (Pomerat—1946 Quart of Phi Beta Phi, 12:1), suggests the interrelation between this effect and the inhibition of one or several enzyme system. Current investigations were undertaken in order to establish whether anti organ sera intervene in the oxidative processes of the cell. Oxygen uptake was measured by the usual Warburg technique. The effect of adding anti organ sera with complement fixation titers ranging from 1:60 to 1:500 were tested at a final dilution of 1:4 and 1:8. Control experiments with corresponding concentrations of normal rabbit serum were carried out simultaneously. In the first series of experiments, anti-rat REIS and rat spleen slices were used. The QO<sub>2</sub> in normal rabbit serum was -11.80, that in REIS, -12.71. As both these figures are very similar and lie in the range of normal oxygen uptake of the spleen, it was concluded that REIS, under these conditions, has no effect upon cellular respiration. Experiments carried out after 3 hours incubation of the rat spleen slices with REIS gave similar results. Likewise, no effect was obtained with concentrations of 1:100, 1:500, and 1:1000, the average QO<sub>2</sub> figures being -10.76, -13.89, and -13.94, respectively. Experiments with chick anti-brain serum showed no inhibition of brain respiration. In these experiments only, brief of chick brain was used and the corresponding average QO<sub>2</sub> was -36.30 with normal rabbit serum as substrate and -33.26 with anti-brain serum. Another series of experiments gave an average QO<sub>2</sub> of -26.78 and -26.03 for normal rabbit serum and anti-brain serum, respectively. Though these figures are somewhat higher than those for the normal respiration of the brain (which possibly might be due to the presence of glucose in the serum), the relative differences between the figures obtained are small enough (10% in the first series) to be ignored.

**Inhibition and facilitation of motor activity by the anterior cerebellum.** F E NULSEN, S P W BLACK, and C G DRAKE (by invitation) *Laby of Physiology, Yale Univ School of Medicine, New Haven, Conn.* Utilization of the inhibitory effect of cerebellar stimulation upon existing motor activity has permitted mapping of the anterior cerebellum as a homunculus representing individual muscles. The cerebral motor cortex or bulbar pyramids are stimulated at regular intervals and the uniform contractions of a single muscle are recorded. Simultaneous stimulation of cerebellar points is then undertaken to establish the location most effective for inhibition of response in that



muscle. Such studies in the cat, dog, monkey, and chimpanzee under dial anesthesia show that the points corresponding to tail, hind-limb, fore-limb, and face musculature have an antero-posterior arrangement in lingula, centralis, culmen, and simplex lobules, respectively. Further localization in the monkey indicates that proximal muscles, as those of the upper arm, are best inhibited by ipsilateral cerebellar points near the mid-line whereas distal muscles, such as the adductor pollicis, are best modified by lateral points in the same lobule. The homunculus for efferent effects thus obtained corresponds to that evolved by Snider and Stowell by observing the localized electrical activity of the cerebellar cortex induced by tactile stimulation.

In each animal, excepting the cat, increasing the frequency of cerebellar stimulation will result in facilitation rather than inhibition of the existing motor movement. The cerebellar homunculus for such facilitation is identical to that obtained when working with inhibition at low frequencies. The dentate nucleus (probably the dorsomedial portion) is involved in inhibition since its bilateral destruction allows only facilitation on surface stimulation, while its direct stimulation yields pure inhibition at all frequencies. Parallel evidence indicates that the fastigial nuclei transmit facilitatory impulses. Analysis of the effects of additional lesions of the brain stem and cerebellar peduncles, combined with the oscillographic evidence of Snider, Magoun, and McCulloch, suggests that both inhibition and facilitation are ultimately mediated through the bulbar reticular formation.

Thus the same cerebellar point which receives tactile stimuli from a localized somatic area will upon electrical stimulation modify motor activity in the same region of the body. Depending upon the frequency of the stimulus, either inhibition or facilitation can be elicited from the same surface point. Evidence suggests that the pathways for the two effects then diverge to travel through different roof nuclei into the suppressor and facilitatory areas of the reticular formation. It is possible that these results of electrical stimulation may mimic the mechanisms by which the anterior cerebellum modifies the movements of an extremity in response to afferent impulses.

**Influence of dibenamine on renal function of the dog.** ERIC OGDEN, *Department of Physiology, University of Texas Medical Branch, Galveston, Texas*. Intravenous dibenamine (20 mg/kg) was given twice to one dog, the doses being separated by a week. The animal immediately appeared nauseated, pale and the jugular veins were empty as in shock. The clearance values were more variable than those immediately before and the urine volumes were diminished. The clearance values observed were as follows: para-aminohippurate before dibenamine 206 (average of 3 days), one

week after first dose 183, one week later 187 ml/min, creatinine, 57.5, 45.8, and 71.6 ml/min. In the last experiment the unexpectedly low PAH clearance allowed the plasma concentration to rise to 10.9 mg/100 ml. The clearance therefore may not be a true measure of renal blood flow, calculated as if in the  $Tm_{PAH}$  range, the  $Tm$  appeared to be 1.19 mg/min. The urine flow was remarkably steady (3.5 to 5.0 ml/min) and was greater than half the creatinine clearance. This indicates that failure of tubular reabsorption accompanied the failure of PAH excretion. At this time the dog was apparently well but optical records of femoral blood pressure read 174/134 with a pulse rate of 168. Six days later the animal was killed. The kidneys had the gross appearance of tubular degeneration, microscopic findings will be reported. The N/P/N was 38 mg per cent post mortem. These findings indicate the need for extreme caution in the use of dibenamine and the matter is being further investigated.

**Further studies on the treatment of experimental renal hypertension with paredrine hydrobromide.** E. A. OHLER (by invitation) and G. E. WAKERLIN, *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago*. We have previously reported (Fed. Proc. 6:171, 1947) the effect of Paredrine HBr (p-hydroxy- $\alpha$ -methyl-phenethylamine) on experimental renal hypertension in seven dogs. Wastl (The Hahnemannian Monthly, Nov., 1942, June, 1944) employed dosages of 0.008 mg to 80 mg/kg of Paredrine HBr in renal hypertensive rats and found the more pronounced antihypertensive effect with the lower dosages. Of a total of 12 renal hypertensive dogs treated with 4 mg/kg of Paredrine HBr orally for periods of from three to seven months, all showed some reduction in blood pressure. One animal gave an excellent response, eight a good reduction, three a fair fall and one a poor response. One neurogenic (debuffered) hypertensive dog showed an excellent reduction. An essential or spontaneous hypertensive dog under treatment at present is exhibiting a good response. Included in the group of eight good responses is one animal which had previously been treated with sodium bromide without effect. One normotensive dog under treatment with 4 mg/kg of Paredrine orally has failed to exhibit any change in blood pressure. Two other amines, choline and Tyramine (2-aminoheptane), have failed to evidence any antihypertensive effect, each in one dog. Animals under treatment with 4 mg/kg of Paredrine orally develop a tolerance to the pressor effect of Paredrine as determined by acute intravenous assay on the unanesthetized trained dog. A cross tolerance was exhibited to Tyramine, tyramine, Benzedrine (2-amino-1-cyclohexylpropane HCl) and Benzedrine but not to epinephrine,

arterenol, ephedrine, renin, hypertensin, or tetra methyl urea

**Renal clearance studies in experimental hypoproteinemic edema** ELIZABETH O'LLARY (by invitation) and SAMUEL A. CORSON, with the technical assistance of OPAL CAIN, CATHERINE ANDERSON and ELINOR FOSTER *Departments of Physiology, School of Medicine, University of Minnesota and Howard University* Edema was produced in 5 trained dogs by means of a low-protein diet and periodic massive plasmaphereses. This method permits the production of a fairly standard degree of edema (as measured by the thiocyanate space) within a period of several days. No anesthesia was used. The interval between the control experiments (at normal plasma protein levels) and the experiments under conditions of edema varied from 18 to 32 days. Renal plasma flow and glomerular filtration rates were measured in the postabsorptive state by determining the clearances of sodium para-amino hippurate (PAH) and creatinine respectively. The solution containing these substances was administered intravenously by means of a constant injection pump delivering 1.5 ml/min. A plasma PAH concentration of 2 mg % or less was maintained by a sustaining dose administered at the rate of 6 mg/M<sup>2</sup>/min following a priming dose of 400 mg/M<sup>2</sup>. A plasma creatinine concentration of about 3-4 mg % was maintained by a sustaining dose of 3 mg/M<sup>2</sup>/min following a priming dose of 400 mg/M<sup>2</sup>. The glomerular filtration rate and the renal plasma flow showed a small but consistent decrease under conditions of hypoproteinemic edema. In spite of a decrease in the plasma colloid osmotic pressure (the plasma proteins decreased from an average value of 6.1 g % to 3.3 g %), there was no observable increase in the renal plasma filtration fraction. On the contrary, a small but consistent decrease was observed (from a normal average value of 33% to 28% hypoproteinemia). Incidentally, our average

values for glomerular filtration rate (98 ml/M<sup>2</sup>/min) and renal blood flow (467) for normal dogs based on creatinine and PAH clearances respectively are in surprisingly close agreement with those reported by Smith (*Physiology of the Kidney*, Oxford U. Press, 1937, page 262) using inulin (94) and diodrast (433) respectively.

**Systolic pressure in the intact, unanesthetized rat** F. OLMS TED (by invitation), A. C. CORCORAN, O. G. ASSER (by invitation) and IRVINE H. PAGE *From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio* An objective means is described for determination of systolic pressure in the intact, unanesthetized rat at normal environmental temperature. Simultaneous graphic records are obtained of the appearance of the pulse in the animal's foot and of the air pressure in an inflatable 'high cuff'. The apparatus consists of animal holder, miniature cuff, adjustable platform for the foot,

an inelastic cloth band around the foot attached to an electric displacement unit (Statham strain gauge YE-16 250), amplifiers, ink oscillograph, and air pressure recorder. The cloth band is a  $\frac{1}{4}$ " wide strip of heavy oiled silk. The ink oscillograph (Brush Development Co.) has a 1" paper strip on which a record of cuff pressure is obtained from a pen moved by a metallic bellows.

The pulse appears as a rough sinusoidal frequency of 300 to 150 per minute superimposed on a transmitted respiratory movement of 60 to 100 per minute. Inflation of the cuff above systolic pressure obliterates the pulse record. The first appearance of the pulse wave as the cuff pressure is slowly lowered is taken as the systolic pressure point. Pressure is read by calibration of the tracing recorded by the pen connected to the cuff and bellows. Forty six preliminary comparisons were made with optically recorded carotid pressure. Carotid pressure, foot pulse, and cuff pressure were photographed simultaneously. The average deviation was  $\pm 5.5$  mm Hg. At arterial pressures ranging from 90 to 150 mm Hg the average of intra arterial readings was 117.1, and of foot pulse readings 115.8 mm Hg.

**Methods for extraction of pressor substances from hypertensive blood** NORMAN S. OLSEN (by invitation), HENRY A. SCHROEDER and MELVIN L. GOLDMAN (by invitation) *Departments of Biological Chemistry and Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri* Extracts were prepared for rat assay of pressor material from the arterial blood of normotensive and hypertensive patients. One volume of arterial blood was drawn directly into 3 volumes of cold 95 per cent ethanol. Further cold ethanol was added to make a total of 10 volumes. Precipitated proteins were filtered off and 1 ml of concentrated HCl added to the filtrate for each 200 ml of original blood. The solute was evaporated to dryness in vacuo below 35°C. The dry residue was extracted with 1 volume of 90 per cent ethanol, filtered and the residue discarded. The filtrate was further concentrated in vacuo to an aqueous residue. This was extracted with petroleum ether which was discarded. The residue was freed of petroleum ether and adjusted to pH 6. This crude extract was found to give pressor effects when injected into rats, but it also contained depressor materials. Using ion exchange resins a separation of the depressor and pressor materials was effected. The latter were found to be present in most extracts of hypertensive blood but not in those from normal subjects. Pierates of the purified material were formed, which on hydrolysis gave pressor effects in rats. From these results it would appear that pressor substances can be extracted from the blood of hypertensive patients.

**Effect of pressure breathing and posture upon**

the respiratory gas exchange and heart rate ARTHUR B OTIS, MITZI SUSKIND (by invitation) and HERMANN RAHN *Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York* Alveolar  $p\text{CO}_2$  and  $p\text{O}_2$ , breathing frequency and volume, and heart rate were recorded at one minute intervals on each of five subjects exposed for 10 minutes to each of the situations listed below The average % change (without regard to sign) of all measurements is listed for each situation (Heart rate, breathing frequency and volume, and alveolar  $p\text{O}_2$  always increased and  $p\text{CO}_2$  decreased) Although such an averaging together of several diverse measurements is a questionable and inexact procedure, it is done here merely to indicate roughly the relative extent of the changes measured for the various situations

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|--|----|
| Supine (Control)                                     | 0% |
| Supine breathing 20 cm H <sub>2</sub> O (+) pressure | 10 |
| Supine pressure breathing with pressure vest         | 5  |
| Supine pressure breathing, legs bandaged             | 10 |
| Tilted passively to 70°                              | 13 |
| Tilted breathing 20 cm H <sub>2</sub> O (+) pressure | 37 |
| Tilted, pressure breathing with pressure vest        | 32 |
| Tilted, pressure breathing legs bandaged             | 31 |

Tilting and positive pressure breathing act synergistically as shown by the fact that the physiological effects of both acting together are greater than the sum of the effects of each acting alone Although the protective effects of a pressure vest or of leg bandages against the combined action of pressure breathing and tilting seem small when judged by the physiological data summarized above, they were sufficient to prevent symptoms of nausea and syncope that appeared in 3 of the 5 subjects when no protection was present

Alterations in fluid and ionic distribution in various patho-physiological conditions R R OVERMAN, C P THARP (by invitation) and A H TUTTLE (by invitation) *Department of Physiology, University of Tennessee, College of Medicine, Memphis, Tennessee* Evidence of severe alterations in ionic distribution in febrile disease as indicated by the dilution of SCN in human and human therapeutic malaria has been previously presented (J Lab Clin Med 31 1170, 1946, Federation Proceedings 6 174, 1947) Statistical analyses of data on 70 human patients with therapeutic malaria indicate that alterations in the distribution of SCN, Na, K, and Cl are not correlated with (a) progressive anemia, (b) height of fever, (c) length of infection, or (d) parasitemia There is a correlation of high significance between the altered cellular ionic levels and the number of paroxysms

If alteration in body temperature occurs in the malarial paroxysm is the etiological factor promoting or allowing cellular permeability change,

then such changes should be reproducible by artificial fever induction Measurement of SCN distribution in a dog during artificial hyperpyrexia (42°C rectal for 40 minutes) revealed an 11% reduction in "extracellular" fluid volume However, if measurement of SCN volume is deferred until the body temperature has returned to normal (8-12 hours later), large increases in the volume of fluid available for SCN dilution occur which are directly correlated with the number of temperature spikes It has been suspected that the effects of fever on the adrenal cortex might allow such permeability alterations The results of simultaneous mannitol and SCN volume measurements in unilaterally adrenalectomized dogs tend to bear out this postulate for, while the mannitol volume decreases, SCN volumes increase as they do in the post-pyrexia state

**Influence of age on carbon monoxide desaturation in man** NELLO PACE, ENRIQUE STRAJMAN (by invitation), and ELAINE WALKER (by invitation) *Division of Medical Physics, University of California, Berkeley* From the work of Roughton and co workers it is found that, following mild exposure, carbon monoxide is released from the blood of man according to a single exponential function of the form  $\text{CO}_t = \text{CO}_0 e^{-kt}$  The rate constant,  $k$ , may be conveniently expressed as the half time of desaturation of CO from the blood,  $\text{CO}_t$ , and the latter has been found to vary from approximately three hours while breathing air to one hour while breathing pure oxygen In the present work,  $\text{CO}_t$  was measured in a series of 14 subjects breathing pure oxygen following the attainment of as much as  $\pm 7$  volumes per cent CO in the blood The  $\text{CO}_t$  was found to vary from 33.5 minutes to 82.2 minutes, and the age of the subjects ranged from 20 years to 65 years A raw correlation coefficient of 0.82 between these variables was calculated, and the slope of the line of regression indicates that at the median age of the group, 42 years, there is a 1.0 per cent longer  $\text{CO}_t$  for each increased year of age This value agrees well with that calculated from the data of Berg (Amer J Physiol 149 597, 1947) for the influence of age on  $\text{CO}_2$  recovery half time following mild exercise It appears that respiration-circulatory changes occur with increasing age, and are reflected in the rate of gas exchange between blood and ambient air

**The influence of tetraethyl ammonium, hepatectomy and selective destruction of the nervous system on vascular reactivity** IRVINE H PAGE, R D TAYLOR and J J REINHARD (by invitation) *From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio* Response to standard doses of adrenalin, nicotine, barium chloride, histamine, angiotensin and renin were studied in normal and anesthetized dogs Two days after destruction of the spinal cord from C<sub>6</sub> caudad re-

sponsiveness is greatly augmented and it becomes difficult to induce renin tachyphylaxis. Administration of  $\text{Et}_4\text{N}$  now has only a slight augmenting effect. Anterior rhizotomy from  $\text{C}_6$  to  $\text{L}_3$ , or ganglionectomy with subdiaphragmatic vagotomy, also increases reactivity, which in such animals is only slightly increased by  $\text{Et}_4\text{N}$ . Removal of the liver in normal animals usually reduces the adrenalin and barium responses, nicotine raises pressure with only occasional lowering while the responses to angiotonin, and especially to renin, are greatly impaired. Even small doses of  $\text{Et}_4\text{N}$  now elicit prolonged pressure fall. The augmentation of pressor substances does not occur. If the kidneys are removed shortly before the liver, the initial response to renin and angiotonin is usually good, but on repeating the dose, the response quickly disappears, not to be restored by  $\text{Et}_4\text{N}$ . Nephrectomy alone increases vascular responsiveness, which is heightened by cord destruction. These preparations are exquisitely sensitive to renin and angiotonin.  $\text{Et}_4\text{N}$  may or may not give further augmentation. Hepatectomy in a dog sensitized by cord destruction decreases the vascular sensitization and the ability of  $\text{Et}_4\text{N}$  to augment. Thus, the nervous system, the liver and kidneys are shown to interplay in determining vascular sensitivity to drugs.

**Susceptibility of X-rayed animals to histamine and to adenosine.** E. E. PANTER and M. C. MOORE (by invitation). *Loyola University School of Medicine, Argonne National Laboratory, and University of Illinois College of Medicine, Chicago, Illinois.* A study was made to determine the susceptibility of X-rayed animals to the blood pressure lowering effects of tissue breakdown products, such as histamine and adenosine. The compounds were infused at a constant rate over 4 minute periods. The amounts required to produce a perceptible decrease (5-8 mm Hg) in carotid blood pressure were determined for the controls and for the X-rayed animals during the first 4 hours after exposure. All animals were anesthetized with pentobarbital before carotid cannulation. Rabbits given a single total body dose of 800 roentgens require only 2-5  $\mu\text{g}/\text{kg}/\text{min}$  of histamine base at 1 to 2 hours after radiation to produce a perceptible decrease in blood pressure. At 3 to 4 hours, however, the amount required, 3-8  $\mu\text{g}/\text{kg}/\text{min}$ , approaches that for the control animals, 10-15  $\mu\text{g}/\text{kg}/\text{min}$ . With adenosine this change in susceptibility is not so apparent. 70-100  $\mu\text{g}/\text{kg}/\text{min}$  for the X-rayed rabbits during the first 4 hours as compared with 90-130  $\mu\text{g}/\text{kg}/\text{min}$  for the controls. Preliminary experiments on dogs given a single total body dose of 400 r show a significant change in susceptibility to both compounds. These findings may be correlated with the following changes occurring in the animal body during the

early period after exposure to X-radiation and, therefore, may be related to radiation sickness: 1) liberation of tissue breakdown products, 2) alteration in the detoxifying powers for such substances, and 3) alteration in the compensatory mechanisms for reducing the effects of such substances.

**Certain physiological reactions to intense sound fields.** H. O. PARRACK and D. H. ELDRIDGE, JR. (introduced by H. M. SWIFLEY). *Aero Medical Lab., Air Materiel Command, Wright Field, Dayton, Ohio.* The response of the ear and other sensory mechanisms to sound fields at a level of 140 to 160 db above 0.0002 dynes per square centimeter will be described. Exposures have been made, at this level, to essentially single frequencies in the range from 5 Kc to 35 Kc. Pain and thermal sensations, hearing loss with suitable exposure frequency, and other sensory reactions are induced. Physiological reactions to intense broad spectrum sound fields will also be described. These fields are of the order of 140 db above the reference level 0.0002 dynes per square centimeter and consist of a continuous sound spectrum from approximately 50 cps to 50,000 cps. Hearing loss occurs together with disturbance of the posture maintaining mechanisms.

**Secretion and excretion of carbohydrate-active adrenal compounds (oxysteroids).** K. E. PASCHKIS, A. CANTAROW, A. A. WALKLING (by invitation), W. H. PEARLMAN, A. E. RAKOFF (by invitation) and D. BOYLE (by invitation). *Jefferson Medical College, Philadelphia.* Study of metabolism of adrenal steroids has so far been mostly concerned with the urinary excretion of androgens and related compounds (19 carbon atom compounds) in humans. Experiments are presented investigating the secretion and excretion of carbohydrate-active compounds (oxysteroids) in dogs and rats using the mouse liver glycogen assay method. No activity was found in peripheral blood of dogs. Blood from the adrenal vein obtained through a London-cannula showed high activity. This confirms data in the literature obtained under less physiological conditions of acute experiments. Active material is found in the urine of dogs but not of rats. After injection of adrenal cortical extract active material is excreted in bile and urine of dogs.

**Influence of temperature on radio-sensitivity in the frog (*Rana pipiens*).** HARVEY M. PATT, MARGUERITE N. SWIFT and ELLA B. TYREE (introduced by AUSTIN M. BRUES). *Biology Division, Argonne National Laboratory, Chicago, Illinois.* There is considerable evidence which suggests that radio sensitivity of tissue is dependent upon certain aspects of its activity (growth rate, temperature, blood flow). Since temperature effects on biological processes are most evident in poikilothermic animals, we have used the frog to study the influence of temperature on survival after

**X-irradiation** In the preliminary experiments reported here animals were given total body X-radiation at 23°C and were kept at different temperatures after irradiation. Survival of experimental animals was corrected for the small mortality observed among non irradiated controls

| Dose     | Temp | No<br>frogs | % survival—weeks after x irradiation |     |     |     |     |     |     |  |
|----------|------|-------------|--------------------------------------|-----|-----|-----|-----|-----|-----|--|
|          |      |             | 1                                    | 2   | 3   | 4   | 5   | 7   | 18  |  |
| <i>r</i> | °C   |             |                                      |     |     |     |     |     |     |  |
| 3000     | 22   | 50          | 90                                   | 63  | 27  | 9   | 7   |     |     |  |
| 3000     | 12   | 10          | 90                                   | 80  | 70  | 70  | 70  |     |     |  |
| 3000     | 5    | 40          | 100                                  | 100 | 100 | 100 | 100 |     |     |  |
| 6000     | 23   | 12          | 100                                  | 0   |     |     |     |     |     |  |
| 6000     | 5    | 12          | 100                                  | 100 | 100 | 100 | 100 | 100 | 100 |  |
| 9000     | 22   | 12          | 100                                  | 0   |     |     |     |     |     |  |
| 9000     | 5    | 12          | 100                                  | 100 | 100 | 100 | 75  | 0   |     |  |

The prolonged survival with low temperature treatment is clearly evident. Some 80-90 per cent of animals kept at 5°C for periods of three to four months after irradiation have survived a dose which is lethal to animals at 22°C within three to six weeks. Experiments are in progress to determine whether the altered radio sensitivity is accomplished merely by a decrease in the rate at which degenerative changes occur or by a more rapid and complete recovery from the effects of irradiation.

**A negative form of the oculo-gravic illusion** J. L. PATTERSON, JR and ASHTON GRAYBIEL (introduced by JAMES V. WARREN) *Naval School of Aviation Medicine and Research, Pensacola and Department of Physiology, Emory University Medical School*. The otolith organs of the non-auditory labyrinth are believed to be the proprioceptors involved in the sense of static position. If a subject remains in fixed position during acceleration on the human centrifuge, the direction of physical force will be changed in relation to him. This force is the resultant of the force due to gravity, centrifugal force and tangential force. In the dark, this situation produces a sensation of bodily tilt in space. A small illuminated visual target, fixed in relationship to the subject, will appear to undergo corresponding displacement (oculo-gravic illusion). The present studies were conducted on the centrifuge with the subject fixed in the sitting position in the dark and continuously observing a free swinging luminous pendulum. This pendulum indicated at any given moment the direction of resultant physical force. Twenty-one experiments on four subjects were done, in each of which the subject was taken from rest to a maximum of 12 RPM and back to a position of rest. At 12 RPM the resultant force was 1.3 G, directed 39°-49° from the true vertical.

Throughout the experiments the direction of the pendulum appeared to remain vertical or very nearly so. This was true even when the pendulum was directed approximately 40° from the true vertical. It is concluded that, in the absence of visual cues and within the limits of the forces produced in these experiments, the apparent vertical is represented by the direction of resultant physical force.

**The effect of oophorectomized dogs' urine extract on gastric secretion** T. L. PATTERSON, J. KAULBERSZ, D. J. SANDWEISS and H. C. SALTZSTEIN (by invitation) *Depts. of Physiology and Surgery, Wayne Univ. College of Medicine and Harper Hospital, Detroit, Michigan*. Urogastrone from normal dogs inhibits gastric secretion in the majority of experiments while a similar extract from hypophysectomized dogs stimulates gastric secretion. In a limited number of studies it was also found that an oophorectomized urine extract inhibited gastric secretion very little if at all. To check the latter findings 41 additional experiments were performed with oophorectomized urine extracts on 5 pouch and 4 fistula dogs. The method of preparation, the yield of the purified extract and the dosage applied were similar to those previously reported. The average output of free HCl after injection of oophorectomized extracts differed only 10.8 per cent from the averages of the control experiments, when administered in the first period an average stimulation of 9 per cent resulted, when given in the second period an increase of 13 per cent was found as compared to the controls. These differences were so close to the variations of the effect of histamine alone at different times that they can not be considered as significant. One might therefore assume that the urine extract from oophorectomized dogs is producing neither inhibition nor stimulation of the gastric juice, following histamine injections. It appears that the ovaries may play a rôle in urogastrone production. However, it is possible that the effect of oophorectomized urine extract is due to the dependence of the ovaries from the regulatory mechanism of the pituitary gland, since the latter has been found to exert some influence on the formation or excretion of urogastrone.

**Instability of motor points and sensory points in the human cerebral cortex** By WILDER PENFIELD and KEASLEY WEICH (by invitation) *McGill University, Montreal, Quebec, Canada*. Sherrington and his pupils analyzed mechanisms by electrical stimulation of the cerebral cortex of anthropoid apes. The human cerebral cortex reacts in a similar manner to such stimulation. There is instability of cortical points, i.e., variation in the response of the same point. In man this may be

shown to apply to sensory points as well as motor. By means of successive stimulations the threshold may be lowered and a given motor response displaced to a distance. This results in deviation and reversal of responses. The limits of sensory and motor stimulative cortex will be discussed.

**The effects of changing the temperature of the normal and denervated nictitating membrane.** JOHN F. PERKINS, JR. and M. C. LI (introduced by E. M. LANDIS). *Department of Physiology, Harvard Medical School, Boston, Mass.* The temperature of the nictitating membranes of cats was changed by means of glass eyes with water circulating through them substituted for the normal eyes. Heights of contraction were determined at water temperatures ranging from 10 to 14 degrees centigrade in 5 degree steps. Upon stimulating the cervical sympathetic, maximal shortening, taken as 100 per cent, occurred at 30 degrees, dropping to an average of 57 per cent at 14 degrees and to 85 at 10. Without electrical stimulation cooling to 10 degrees produced 61 per cent shortening, warming to 14 degrees gave minimal, i.e. zero per cent shortening. Evidence from epinephrine dose-response curves suggests destruction of sympathin at the higher temperatures rather than decreased contractility. Effects of cooling were studied by suddenly, or gradually, lowering the temperature of the water from 37 degrees by varying amounts. The denervated nictitating membrane contracted in jerky, stepwise fashion when cooled, in contrast to the smooth contraction of the normal. When heights of contraction were plotted against degrees of sudden cooling the shape of resulting curves was different from that of dose-response curves for epinephrine, suggesting separate response mechanisms. When cooling and epinephrine were tested simultaneously the response equalled the sum of the two effects when tested separately, no true synergism existing. In two of four experiments the denervated nictitating membrane showed true sensitization to cold, contracting in response to smaller drops in temperature than the normal and showing greater contractions with a given drop in temperature for all but maximal contractions.

**Effect of insulin upon the in vitro glucose utilization and glycogenesis of the diaphragm of normal and pituitarectomized rats.** Adaptation of this technique as an assay for serum content of insulin and anti-insulin substances. M. PERLMUTTER (introduced by G. W. THORN) and R. O. GREIF. *Department of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, and the Harvard School of Dental Medicine, Boston, Mass.* Gemmell has shown that insulin increases the glucose utilization and glycogen synthesis in the isolated rat diaphragm. Using the same in vitro technique, studies were made of the insulin effect on the diaphragm of normal and

pituitarectomized rats. The experiments were done on the fourth and fifth day after hypophysectomy. The insulin effect on the glucose utilization was not decreased in the hypophysectomized tissue. By modifying this technique, an assay for insulin content of human serum has been devised. Glycogen synthesis in a mixture of normal serum and 0.5 per cent glucose in Krebs phosphate medium was  $0.32 \pm 0.011$  (S.E.M.) mg per cent. Further work is being done, comparing the effect of sera from normal and hypoglycemic humans to determine the value of this test in the diagnosis of hyperinsulinism. The addition of 0.01 units of insulin per ml. of serum in vitro or the injection of 20 units of crystalline insulin intravenously into the human significantly increases the glycogenetic effect of the serum. By the use of this method, a search for anti-insulin factors in the sera of insulin resistant diabetics and patients with Cushing's syndrome is being made.

**Effect of graded degrees of muscular tension on human heart rate.** JOHN L. PETERS (by invitation) and W. HORSLEY GAYT. *Pavlovian Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins Hospital.* These experiments were designed to show the changes in heart rate with accurately measured muscular tensions in humans. Previous studies from this laboratory revealed that even slight emotional states—those produced by a conditional stimulus for a small amount of food (2 gms.) or for faradic shock—are accompanied by cardiac acceleration. How much of this results from increased muscular tension, how much from central nervous excitation independent of peripheral tension? In 9 normal subjects exerting a) maximal tension with left hand on a dynamometer 5 seconds, b) maximal tension 30 seconds, c) one sixth maximal tension for 2 minutes, there was 1) great individual variation [56-95 control, 68-116 with a), 90-136 with b), 66-117 with c)], 2) accelerated heart rate with a) and b) [from 82 control to 97 test with a) and from 85 control to 112 test with b)]. With one sixth maximal tension no change in heart rate occurs during the first 30 seconds, a moderate increase during the last 90 seconds coincided with fatigue. The muscular tension produced with one sixth of maximal is nearer the muscular tension during a conditional reflex—considerably less than our maximal tension. Respiratory changes were much less than those accompanying a conditional reflex having a fairly strong underlying motivation.

From these and other experiments we conclude that an increased heart rate occurring with a salivary or even with a motor conditional reflex is much more a result of central nervous excitation than of any accompanying peripheral muscular tension.

**Beneficial effects of calcium chloride in fluoride**

**poisoning** J H PETERS (by invitation), L GREENMAN (by invitation), and T S DANOWSKI *From the Department of Research Medicine and the Renziehausen Foundation, University of Pittsburgh, School of Medicine* 10 cc of a 5 per cent solution of sodium fluoride in water was administered by gavage to immature female rats weighing 60 to 115 grams. Without treatment death occurred in 24 hours or less in 75 to 95 per cent of the animals in a series of experiments. The introduction into the stomach of 30 cc of a 4 per cent solution of calcium chloride in water just prior to, or within 5 minutes after, the sodium fluoride reduced the mortality to less than 10 per cent. A delay of 10 minutes in the administration of the calcium chloride to fluoride-poisoned rats reduced significantly the efficacy of this treatment. Tetany, a frequent though not invariable manifestation in the rats given sodium fluoride, disappeared with calcium chloride therapy in all but the moribund animals. The beneficial effects of calcium chloride in fluoride poisoning are related, in all probability, to the removal of the fluoride ion from solution as the highly insoluble salt, calcium fluoride. At the same time the hypocalcemia and tetany which develop in fluoride poisoning are corrected.

**Direct blood pressure recording in man** L H PETERSON and G C RISMAN (introduced by H C BAZETT) *Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.* An innocuous, mobile, practical blood pressure recording device has been developed by combining with a Lilly capacitance manometer the catheterization technique described a year ago and an ink writer—d.c. amplifier couple. The response is critically damped and linear from d.c. to one hundred cycles up to 300 mm Hg. Intra-arterial pressure pulses have been recorded from the human continuously under a wide variety of circumstances including surgical and other clinical procedures. To date over seventy-five patients have been followed through anesthesia and surgery. Congenital heart defects and normal humans under physiological stresses have also been studied. The technique does not interfere with the patient's comfort or the surgeon's convenience.

Correct manometric records have been obtained continuously for up to ten hours without clotting or flushing. One individual can accomplish the entire procedure. Calibrations and base line determinations can be made at will. Under such circumstances it is easy to see the wave shape and correct pressures of each beat as it occurs. Deductions can be made, when using each individual as his own control, whether changes in pressure or pulse shape are due primarily to imbalance of cardiac output, peripheral resistance or arterial distention. Such immediate knowledge greatly aids correlation of causes and effects. Surgery, treat-

ment or experimental procedure can be modified according to the effects observed and valuable time may be saved both in surgery and in repetition of experiments. Data are presented to demonstrate specific examples of such values.

**The effect of adrenalectomy or evisceration on cyclopropane induced cardiac arrhythmias in cats** FRANK L PETTINGA (by invitation) and J W STUTZMAN *Department of Pharmacology, Boston University School of Medicine* After induction with a cyclopropane-oxygen mixture unanesthetized cats were intubated and maintained in deep surgical anesthesia by rebreathing a mixture of 28 per cent cyclopropane in oxygen from a 100 liter reservoir. The beam of the electrocardiograph (Lead II) was under constant observation. Records were taken every two minutes and when a change of rhythm was observed. Control experiments consisted of recording the cardiac response during 30 minutes of cyclopropane anesthesia. On a subsequent day the animals were again placed on the anesthetic mixture. As soon as a record of cardiac arrhythmias was obtained bilateral adrenalectomy or abdominal evisceration was performed. The finding of Allen et al. (*Anesthesiology* 3: 530, 1942) was confirmed that without exogenous epinephrine cats develop cardiac arrhythmias during cyclopropane anesthesia. The arrhythmias consist of ventricular premature contractions, bigeminal rhythm, and ventricular tachycardia. Bilateral adrenalectomy caused the preexisting ventricular tachycardia to revert to S-A rhythm within two minutes in 5 of 6 animals. In the sixth a normal rhythm followed evisceration. Removal of the spleen, gastrointestinal tract and accompanying mesentery from cardia to rectum abolished the ventricular arrhythmias in 5 of 7 cats. Visceral arteries were ligated within 2 cm of the aorta. Adrenalectomy restored a normal rhythm in one of the remaining two, and splenectomy in the other.

**Variations with age in neutral steroid excretion of men** GREGORY PINCUS, LOUISE ROMANOFF (by invitation) and JAMES CARLO (by invitation) *The Laboratories of the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts* Data have been collected on over one hundred normal, healthy men of various ages on the urinary 17-ketosteroid excretion. For basal (sleep) urines there is no significant difference in the mean excretion values between the 3rd, 4th and 5th decades of life. A decline in mean excretion values is apparent at the 6th decade which continues on into the 9th decade. The diurnal rhythm of excretion of 17-ketosteroids is scarcely altered in the older age groups. Data on the urinary excretion of neutral reducing lipids reveal no change in mean basal values through the 8th decade. A more irregular diurnal rhythm of excretion appears in the older

men Since both of these measures reflect adrenocortical secretion it is concluded that those physiological activities influenced by the neutral reducing lipid precursors are constantly maintained in healthy men whereas the physiological activities influenced by 17-ketosteroid precursors diminish in the later decades of life

**The effect of vagotomy on the secretion of pancreatic juice after the ingestion of various food-stuffs (Preliminary Report)** I J PINCUS (by invitation), J E THOMAS and P O LACHMAN (by invitation) *Jefferson Medical College, Philadelphia, Pennsylvania* In view of the current interest in the effect of vagotomy on gastro intestinal function, it was thought timely to present this report Feeding experiments were performed on three dogs to determine the normal pancreatic response to various foodstuffs Milk, meat, bread and olive oil were fed in quantities which represented approximately equal amounts of total solids The volume and specific gravity of the pancreatic juice was determined and the nitrogen output calculated These experiments were then repeated in one animal after a successful transthoracic vagotomy Following vagotomy, the volume of pancreatic juice was reduced to a significant degree The average hourly secretion for the five hours after feeding 250 ml of milk dropped from 18.6 to 7.8 ml, when 55 grams of meat was fed, it decreased from 23.4 to 9.1, whereas 35 ml of olive oil produced an hourly secretion of 17.9 ml before vagotomy, it caused an hourly flow of only 11.1 ml after vagotomy, 50 grams of bread, which had caused an hourly flow of 23.1 ml, produced only 8.2 ml after vagotomy The specific gravity of the pancreatic juice was reduced, and the total nitrogen output was markedly diminished

It would seem that after vagotomy, the secretion of pancreatic juice in response to food is decreased in amount, the enzyme concentration which parallels the specific gravity is low, and the nitrogen output is markedly reduced It may be that some of the complications observed when vagotomy is used as a therapeutic measure are produced by this effect on pancreatic function

**The reabsorption and excretion of bicarbonate in normal man** By R F PITTS, J L AYER and W A SCHIESS *Dept of Physiology, Syracuse University College of Medicine, Syracuse, N Y* In a series of 14 experiments on 3 normal male subjects in whom plasma bicarbonate was elevated to 38 mM per liter by infusing sodium bicarbonate, from 2.6 to 3.0 mM were reabsorbed per 100 ml of glomerular filtrate The excess appearing in the filtrate was excreted At plasma concentrations of 13 to 23 mM per liter (following ingestion of ammonium chloride) reabsorption of bicarbonate was 99.9% per cent complete Thus the normal renal threshold for bicarbonate in our experiments varied

between 21 and 26 mM per liter It is suggested that the mechanism for the reabsorption of bicarbonate in the distal tubular segment is the same as that which converts buffer salts into free titratable acids by the exchange of hydrogen ions for fixed base If this same exchange were effected for base bound by bicarbonate, one would predict first, that the partial pressure of carbon dioxide in the urine (i.e. concentration of free carbonic acid) should increase in proportion to the rate of distal tubular reabsorption of bicarbonate, and second, that the rate of excretion of titratable buffer acid should be inversely related to the rate of distal tubular reabsorption of bicarbonate, i.e. carbonic acid should substitute for buffer acid The experimental data are in accord with both predictions It is probable that the proximal tubular mechanism reabsorbs bicarbonate isototically, i.e. in proportion to water If true, the volume of fluid reabsorbed in the proximal segment must be inversely related to plasma bicarbonate concentration

**Blood and urine levels of theophylline after intravenous injection** A J PLUMMER (introduced by GEORGE L MAISON) *Department of Pharmacology, Boston University School of Medicine* A method for the determination of theophylline has been developed and will be reported in detail elsewhere Theophylline is extracted from a trichloroacetic acid blood filtrate, precipitated as a copper compound in methyl alcohol solution and determined by titration of the iodine liberated from a soluble iodide by the copper compound The method is sensitive to 0.1 mg per 100 cc blood It has been applied to blood and urine Theophylline was determined in the blood of five dogs after the intravenous injection of 10 mg per kg of theophylline with ethylene diamine at intervals of five, thirty, sixty, and ninety minutes after the injection The levels, expressed in mg theophylline per 100 cc of blood are as follows: at 5 minutes 0.76 to 1.10, at 30 minutes 0.43 to 0.58, at 60 minutes 0.16 to 0.30, at 90 minutes 0.10 to 0 Theophylline was detected in only one animal's blood after 90 minutes The theophylline in the urine was determined in catheterized specimens taken at thirty, sixty and ninety minutes after the intravenous injection During the first thirty minute period the average excretion was 0.062 mg per kg, during the second thirty minute period 0.046 mg per kg and during the third period 0.036 mg per kg During the 90 minute period 1.8% of the injected drug was excreted

**Venous pressure in the human leg during exercise and in various positions** ALBERT A POLLACK and EARL H WOOD *Section on Physiology, Mayo Clinic, Mayo Foundation, Rochester, Minnesota* Direct and continuous measurement of the pressure in the great saphenous vein at the ankle in eleven normal subjects in the supine, sitting and



standing positions and while the subjects were walking on a treadmill was made by means of a strain gauge manometer connected to a polythene tube which was inserted in the vein through a no 17 needle. The average pressure in the supine position was 11.6 (7-15.9) mm of mercury. In the sitting position, the average pressure was 55.9 (45-67.5) mm of mercury and in the resting standing position was 86.8 (78.5-92.6) mm. Resting venous pressure at the ankle in the various positions studied was sufficient to support a column of blood up to the third thoracic interspace at the sternum. Taking a single step produced an average initial rise of 10 (-3 to 28) mm of pressure even before the heel was lifted from the floor. An average fall of 51.8 (28-80.5) mm of mercury occurred while the foot was off the floor. As the heel returned to the floor, there was a slight rise in pressure, followed by another fall to the previous low level as the heel settled on the floor (and the body weight was redistributed). As the subject stood quietly after taking the step, the pressure returned to the control level over a period of 22.9 (11-37) seconds. While the subjects were walking on a treadmill at 1.7, 2.6 and 3.3 miles per hour, the pressure fell in all subjects in the first four to twelve steps to an average mean stable value of 22.3 (11-30.6), 24.3 (8.5-38) and 23.5 (10-43.2) mm of mercury respectively and returned to the control level in 31.2 (8-57) seconds after the walk. Inclining the treadmill to 20 degrees made no difference in the fall of pressure during the walk.

**The toxicity of various stimulants of nervous tissue as revealed by tissue culture technique**  
J. PAINTER and C. M. POMERAT. *Tissue Culture Laboratory of the Department of Anatomy and the Psychopathic Hospital, Medical Branch, The University of Texas, Galveston, Texas*. A technique has been devised to observe the outgrowth of nerve fibers and glial elements from explants of spinal cords of 9 day chick embryos in hanging drop preparations. The toxicity of various barbiturates has been studied with this method (Proc Soc Exper Biol and Med 63:322 1946).

Total inhibition of fiber outgrowth resulted from the inclusion of 500  $\gamma$ /ml of benzedrine sulfate while a few short fibers were seen in medium containing 250  $\gamma$  and cultures with 150  $\gamma$ /ml of this drug resembled the controls. Metrazol was studied with the use of a 10% solution ordinarily employed for clinical purposes (Bilhuber Knoll Corporation). Total inhibition was obtained with a 1:8 dilution of 1:10 (10,000  $\gamma$ ). Picrotoxin proved remarkably non-toxic. Outgrowth from explants in a medium containing 3.75 mgs/ml appeared uninjured. The migration of fibers was not damaged in medium containing 1000  $\gamma$ /ml of camphor tetrazol but the addition of 1250  $\gamma$ /ml proved totally inhibitory. Crystalline and protamine zinc insulin at 10

$\gamma$ /ml permitted outgrowth of fibers resembling corresponding untreated control cultures for the first 24 hours, but by 48 hours it was evident that these elements were arrested. Stained preparations revealed only fibers of relatively large diameter, the dense network of fine fibers being almost completely eliminated.

**Uropepsin excretion in man** C. J. PODORE (by invitation), R. H. BROTH-KAHN (by invitation) and I. ARTHUR MIRSKY. *May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio*. Studies have been undertaken to determine some of the various factors that influence the rate of uropepsin excretion in man. It was first essential to demonstrate that the activity of uropepsin in the urine specimens as they are tested in the laboratory reflects the true concentration of this enzyme as it is excreted in the urine. The stability of uropepsin was confirmed and no uropepsin inhibitors were found in urine. The urine from numerous normal subjects was collected over a 24 hour period for several weeks without interruption. In many cases the urine excreted during the hours of activity was collected separately from that excreted during sleep. No correlation could be observed between the excretion of uropepsin and the acidity of the urine or the rate of urine excretion. Uropepsin was excreted at a fairly constant rate over a 24 hour period and was not influenced by urine volume so that it was the total amount of uropepsin appearing in the urine rather than its concentration that characterized its excretion. Ordinary fluctuations in daily diet failed to produce any marked variation in excretion. Nocturnal excretion in general was somewhat lower than excretion during the day but much overlapping was observed. Each subject excreted uropepsin in a fairly characteristic pattern with but fairly small daily fluctuations. Children were characterized by a lower uropepsin excretion than were adults. The excretion of patients with gastric lesions was compared with those of normal subjects. The great majority of patients with benign peptic ulcer fell into a group that was characterized by a higher daily mean uropepsin excretion than that of the normal. Fluctuations about this mean were greater in the ulcer group than in the normals. Patients with malignant ulcers, as verified by pathological examination, were characterized by a somewhat lower uropepsin excretion than the normal subjects.

**Variation of total circulating hemoglobin and reticulocyte count with season and following hemorrhage** R. L. POST (by invitation) and C. R. SPEALMAN. *University of Pennsylvania, Medical School (Physiol Dept)*. Total circulating hemoglobin was determined by the carbon monoxide method using a filter photometer on four men at about two week intervals from June 1946 through August 1947. Reticulocytes were counted at similar

intervals from December 1946 through August 1947. The mean monthly outdoor temperature ranged from 30°F to 70°F in this period. During the summer of 1946 increases in total circulating hemoglobin from 6 to 11% were observed but in 1947 no significant increase was found. Similarly no increase in reticulocyte count was found in 1947. These results need not be inconsistent with reports in the literature of a reticulocytosis in the spring and an increase in total hemoglobin in hot weather because these subjects spent most of their time indoors. To determine the reticulocytosis produced by a sudden small deficit of hemoglobin four other subjects were bled 10% of their blood volumes and total hemoglobins and reticulocyte counts were determined almost every day for more than two weeks. The hemoglobin deficit was approximately one-half restored in two weeks. The reticulocyte count rose almost linearly from a normal of 7 per 1000 rbc to a peak of 18 per 1000 rbc in 14 days.

The reticulocyte count cannot be taken as directly proportional to hemoglobin production under these circumstances. On the basis of our data it would appear that either the maturation time of the reticulocytes must increase or that a higher proportion of red blood cells leaving the marrow must be in the reticulocyte stage following such a hemorrhage.

**Action of narcotics on synapses compared to action on axons in sympathetic ganglia.** J. M. POSTERNAK (by invitation) and M. G. LARRABEE, *Johnson Foundation, University of Pennsylvania*. The action of narcotics on synaptic functions were compared with effects on axonal conduction by recording impulses transmitted through perfused stellate ganglia of cats. The postsynaptic action potential in the inferior cardiac nerve following preganglionic nerve stimulation measured the number of cells responding to synaptic excitation, the action potential of the cervical sympathetic trunk measured the number of type B fibers conducting impulses through the stellate ganglion without synapse. Chloroform and ethyl ether exhibited preferential action on the synaptic pathway, which was depressed by concentrations about  $\frac{1}{3}$  those which equally depressed conduction over B-fibers. Both narcotics caused detectable effects at concentrations equalling those in blood for human surgical anesthesia. By contrast, ethyl alcohol (previously reported) and methyl alcohol had no preferential action on synapses. Actually axonal conduction was depressed in concentrations about  $\frac{1}{2}$  the concentrations equally depressing the postsynaptic response. Higher aliphatic alcohols exhibited a pronounced differential action, octyl alcohol depressing the synaptic pathway in  $\frac{1}{2}$  the concentration required for equal effect on B-fibers. This preferential action developed progressively

in the aliphatic series ( $C_1$  to  $C_6$  and  $C_8$ ), the ratio of required concentrations being a linear logarithmic function of the number of carbon atoms.

For a given effect on the synaptic pathway, thermodynamic activities were nearly equal for ethyl ether, chloroform and the alcohols, while molar concentrations varied nearly 6000 fold. For effect on B-fibers, the thermodynamic activities increased ten fold with number of C atoms in the alcohols, the activities for chloroform and ethyl ether being intermediate.

**A simple calorimeter for simultaneous determination of heat production and heat loss in laboratory animals.** LAWRENCE R. PROUTY (by invitation), Martha J. Barrett (by invitation) and JAMES D. HARDY, *From the New York Hospital and the Department of Physiology, Cornell University Medical College, New York, New York*. The calorimetric technique developed by Day and Hardy for premature infants has been improved to give a rapid method of simultaneous measurement of respiratory metabolism and heat loss in animals. Expensive equipment and involved procedures heretofore required for direct heat determinations have been largely eliminated. Heat production and heat loss in calories per hour can be measured in single periods with an accuracy of  $\pm 2\%$ . A single observer using half hour periods can operate the entire apparatus. The calorimeter consists of two concentric copper cylinders separated by a 10 mm air layer. Air is circulated through the inner cylinder (the calorimeter proper). Thermocouples are placed at intervals over the surface of each cylinder and in the ingoing and outgoing air stream apertures. Within and insulated from the inner cylinder are a wire mesh floor, skin, muscle and rectal thermocouples and electrocardiograph leads. Activity of the animal is recorded from a pneumograph attached to the suspended calorimeter and from electrocardiograph changes. Direct calorimetry is based on the measurement of thermal gradient between the two copper cylinders, the outer of which is cooled to room temperature by an electric fan. Correction is made for vaporization and calorimeter storage under non equilibrium conditions. The calorimeter is sufficiently versatile to be used with either closed or open circuit method. We found the open circuit, gravimetric method of Haldane sufficiently accurate for general use. Where small quantities of oxygen and carbon dioxide are present the gasometric method of Barker is more applicable.

**Man's respiratory response during acclimatization to high altitude.** HERMANN RAHN and ARTHUR B. OTIS, *Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York*. The changes in alveolar air composition during acute exposures to high altitude are compared to those recorded in the literature for

acclimatized individuals. A diagram is presented which allows one to visualize the simultaneous alveolar oxygen and carbon dioxide pressures as well as the alveolar ventilation at any altitude and to predict the magnitude of the changes in these three quantities during the process of acclimatization. The prediction for changes in alveolar air were verified by exposure of three subjects to 9500 ft for several weeks.

With the assumption that the blood pH returns to normal when acclimatization is complete, one can estimate that the alkali reserve at this altitude is lowered approximately 20%. If the pH has a regulatory action upon the ventilation then the reduced alkali reserve should make the respiratory system more sensitive to equal increments in  $\text{CO}_2$ . This is indicated by (1) a greater ventilation response to 22 and 34 mm  $\text{pCO}_2$  in the inspired air after acclimatization, (2) a smaller increment in alveolar  $\text{pCO}_2$  necessary to achieve the breaking point after breath holding when pure oxygen was breathed at altitude and, (3) by reduction in breath holding time when breathing pure oxygen at altitude.

**Dynamical and electrical features of human isolated voluntary muscle in isometric and isotonic contraction.** H. J. RALSTON, J. R. CLOSE (by invitation), V. T. INMAN (by invitation) and B. FEINSTEIN (by invitation). *Dept. of Physiology, College of Physicians and Surgeons, San Francisco, and the University of California School of Medicine and College of Engineering, San Francisco and Berkeley.* Mechanical and electrical responses of essentially isolated voluntary muscle have been studied in amputees having cineplastic muscle tunnels. Stimulation of the muscles was in all cases voluntary. Isometric length-tension diagrams reveal the same basic features as those shown by frog muscle. Since force developed varies with length of muscle, no correspondence between mechanogram and electromyogram exists, except at a given length of muscle. Isometric contraction times are much greater than those in artificially stimulated muscle-nerve preparations, in some cases exceeding one second. The slow development of both force and electrical activity in voluntary contraction indicates that voluntary response is at first fractional. At a given length of muscle, forces developed in isotonic contraction are only a few per cent of those developed in isometric contraction. Velocities of free contraction as great as six times the resting muscle length per second have been observed.

The effect of eserine on the absolutely and relatively refractory period of turtle heart strips. ROBERT W. RAMSEY, GEORGE FISCHER (by invitation) and MARIE LOUISE FLICKER (by invitation). *Department of Physiology and Pharmacology, Medical College of Virginia, Richmond, Virginia.* Turtle heart strips (ventricle) were equilibrated

overnight in Ringer's solution, and on the following day stimulated electrically at a constant rate of 6 per minute. The temperature ranged from 25 to 30°C. Short condenser shocks ( $\text{cr} = 5$  milliseconds) were applied at various intervals between two successive ten second responses and the voltage required to cause an additional response recorded. Adequacy of response to the interpolated shock was judged by the appearance of a second action potential. When the voltages of the interpolated shocks are plotted against time measured from the preceding shock, the resultant curve maps out both the absolutely and the relatively refractory periods. Such curves were obtained first in Ringer and then after application of the Ringer eserine solution. In a few experiments the curves were redetermined after washout of the drug by Ringer's solution. The concentrations of the eserine solutions used (either as salicylate or sulfate) ranged from 1/100,000 to 1/10,000. In the majority of instances eserine slowed the rate of recovery of the threshold to its initial value and thus prolonged the relatively refractory period. There was no experiment (out of 18) in which the drug shortened the relatively refractory period. The drug did not significantly affect either the absolute refractory period or the initial threshold.

**Ventricular isometric relaxation phase as measured on the electrokymogram.** EDWARD F. RANDAK (by invitation), BERT R. BOONE (by invitation), GEORGE F. ELLINGER (by invitation), and M. J. OPPELHEIMER. *Dept. of Physiology, Temple Univ. School of Medicine and Heart Disease Control Section, U. S. Public Health Service, Philadelphia, Penna.* The development of the electrokymograph (Am. Jr. Roentgen and Rad. Therapy 57:409, 1947), has provided a new method for studying the pulsations of the heart and great vessels on the intact human subject. Clearly recorded serrations following the ventricular ejection limb appear to provide an easy means of measuring the duration of the isometric relaxation phase. Little importance has been given this phase in past investigations. Wiggers and Burstein, utilizing optical venous and arterial tracings on man, have provided some figures with which those of our direct method are in close agreement.

|                      | Range       | Average |
|----------------------|-------------|---------|
|                      | seconds     | seconds |
| Wiggers and Burstein | 0.037-0.136 | 0.076   |
| Randak et al.        |             |         |
| 95 normals           | 0.060-0.160 | 0.113   |
| 22 abnormal          | 0.100-0.220 | 0.178   |

There is little difficulty in measurement of this phase on better than ninety-five per cent of the subjects. The duration appears to be independent of heart rate (between rates 70-115), and inde-

pendent of age, sex and blood pressure. Ninety-seven per cent of the normals were found to have durations in the range of 0.060 to 0.140 seconds, inclusive. This measurement was taken on twenty-two patients with heart disease, consisting of myocardial infarcts, bundle branch blocks, and hypertension. Significant increases in duration of this phase were noted in a majority of the cases. Eighty-six per cent were found to have durations in the range of 0.150 to 0.220 seconds, inclusive. This prolongation in isometric relaxation has not been found to be characteristic of any one pathologic condition studied. These results suggest that more importance may be given to this phase of the ventricular cycle, and further investigation into the physiologic factors concerned is indicated.

**Effects of the ablation of the pituitary, adrenals and typhoid on liver regeneration, nucleic acid partition, etc.** DAVID RAPPORT, ATTILIO CANZANELLI and RUTH GUILD (by invitation). *Department of Physiology, Tufts College Medical School, Boston*. Partition of ribonucleic acid (PNA) and desoxyribonucleic acid (DNA), total solids, total N, and the amount of liver regenerated were studied in rat livers before partial hepatectomy, in which about  $\frac{2}{3}$  of the liver was removed, and after 96 hours of regeneration. In all cases, the control and experimental animals received approximately the same amount of a synthetic diet. The findings below are to be construed as a comparison with results obtained on animals with intact endocrine glands. (1) *Pituitary*. Hepatectomy was performed 4 to 7 days after hypophysectomy. In the pre-hepatectomy liver there was a small increase in PNA, but no other significant findings. In the regenerated liver, DNA was significantly increased, and the amount of liver regeneration was reduced to  $\frac{1}{2}$  of the control. (2) *Adrenals*. PNA was increased both in the pre-hepatectomy liver (10 days after adrenalectomy) and in the regenerated liver. There was a tendency towards reduction in regeneration. When the animals were subjected to a temperature of 31°C during the entire experiment, the decrease in regeneration became much more marked. (3) *Thyroid*. Six weeks after thyroidectomy, the pre-hepatectomy liver showed a reduction in PNA and total N, the regenerated liver showed only tendencies which were not statistically significant. However, animals fed desiccated thyroid showed by comparison with the normal, and particularly with the thyroidectomized animals, a significant rise in PNA of both the pre-hepatectomy and the regenerated liver, as well as an increase in the amount of liver regenerated.

The results seem to indicate independently acting effects on the liver by the three glands studied.

**The partial purification and some properties of an adaptation-stimulating principle from yeast** JOHN M. REINER and S. SPIEGELMAN (Department

of Bacteriology and Immunology, Washington University School of Medicine, St. Louis, Mo.) Recent studies of adaptive enzymes in yeast have suggested the hypothesis that enzymes are formed by self-duplicating cytoplasmic units. The existence in adapted cells of materials having some of the properties to be expected from such units was reported earlier (Cold Spring Harbor Symposia on Quantitative Biology, 11: 256 (1946); J. General Physiology, 30: 755 (1947)). We now have a reproducible procedure by which an adaptation stimulating principle can be prepared from galactose-adapted yeast cells, and separated from an as yet unidentified inhibitor. The active principle does not appear to be dialyzable. It is inactivated by brief exposure to cold acetone and by prolonged exposure to cold alcohol, but resists heating at 60°C for as long as 15 minutes. Its activity is unaffected by desoxyribonuclease, ribonuclease, pepsin, trypsin, chymotrypsin, or papain. Incubation with protein denaturants such as urea inactivates the principle. The denaturants also inhibit normal adaptation, but the cells recover and eventually adapt. When the active principle is added to cells simultaneously with the denaturant, no inhibition is observed. Addition of the adaptive substrate prior to or together with the denaturant also has a protective effect. An important characteristic of this material is its extreme instability in the absence of the specific adaptive substrate. Usually all steps of a preparation are performed in the presence of the substrate (1% galactose). Omission of galactose or substitution of glucose leads to disappearance of activity. No desoxyribonucleic acid was detected in this material. Determinations of nitrogen, phosphorus, and ribose are consistent with the supposition that a nucleoprotein may be present. Prolonged treatment with chloroform to remove extraneous protein does not affect the activity, and the residue still contains large amounts of protein and has an N-P ratio too high for a nucleic acid. The possibility that the active material is the adaptive enzyme itself has been ruled out by experiments in which it was added to active extracts from unadapted yeast, which have been shown to possess all the non-protein components necessary for the adaptive fermentation (Archives of Biochem., 13: 113 (1947)). The combination fails to ferment galactose.

**Effects of hemorrhagic shock on hepatectomized and bilaterally nephrectomized, hepatectomized dogs** J. J. REINHARD, JR. (by invitation), O. GLASSER (by invitation) and IRVING H. PAGE. *From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio*

Hepatectomized and bilaterally nephrectomized hepatectomized dogs were subjected to a standard hemorrhagic shock. The response of these animals did not differ significantly from that of animals

with livers and kidneys intact. When the dogs' own blood was therapeutically restored by intra arterial transfusion the blood pressure increased and was maintained at the pre shock level for 2 to 3 hours. Responses to adrenalin in the pre shock control period and the post shock control period were equal.

Other hepatectomized dogs were given 250-300 cc of blood from animals in hemorrhagic shock whose livers were intact. In order to keep the blood volume constant, the blood from the shocked donor was transfused into one leg while an equal amount of blood of the hepatectomized dog was removed from the other leg. Some of the donor animals had intact kidneys and some had been bilaterally nephrectomized prior to shock. The hepatectomized recipients showed no toxic effects of the transfusion and the blood pressure remained constant.

To demonstrate further the lack of toxic effects following transfusion, the hepatectomized recipients were bled to produce shock. The response to bleeding was identical to that of hepatectomized dogs which had not received blood from a shocked donor.

Thus, it is seen that the absence of the liver does not change significantly the response of dogs to hemorrhagic shock.

**Some cutaneous responses to 'reflex heating'**  
WALTER C. RANDALL, DOUGLAS E. SMITH, and ALBRICK B. HERTZMAN, *Department of Physiology, St. Louis University, School of Medicine*

Simultaneous observations were made upon the cutaneous blood flow, the skin surface temperature, and the number of functional sweat glands on the forehead, cheek, forearm, and finger pad while normal male subjects were exposed to "reflex heating" experiments. Immediately following exposure of the lower extremities and the lower trunk regions in a hot water bath (38-44), blood flow and skin temperature of the finger pad quickly increased to high levels. Simultaneous sweating responses on the finger pad showed either no change, or a slight increase. In all other areas studied a definite time lag followed the exposure to heat before increases in blood flow, temperature and sweating were observed. Increases in sweating and in blood flow generally occurred approximately together, although in some experiments the sweating responses appeared somewhat earlier than vasodilatation. Relative flow and sweating responses in the four body areas both in the normal and the heat dilated state, are described and compared with maximal responses induced by direct stimulation by histamine and mecholyl.

**Physiological protection of the extremities from severe cold.** S. I. RAPAPORT (by invitation), E. S. FETCHER, and JOHN F. HALL (by invitation). *The 1600 Laboratory, Wright Field, Dayton, Ohio*

Hand and foot temperatures in the cold can be made primarily dependent upon the state of thermal balance of the body, and nearly independent of ambient temperature and insulation.

Forced internal ventilation of clothing permitted control of the thermal state of the body through regulation of a hot air supply (see Fletcher, Rapaport, and Hall, this issue). The hands and feet were excluded from the ventilating circuit, receiving heat only from their blood supply. The insulation of the footwear and gloves, calculated in clo, was 2.6 and 0.9 respectively, that of the body clothing was 2.5 clo. The effect upon hand and foot temperatures of variation of the heat supplied to the body was studied at 0°F and -30°F. It was found, in three subjects, that normal hand and foot temperatures were maintained when approximately 180 kg Cal/hr were supplied at 0°F, and 280 kg Cal/hr at -30°F, i.e., when the body did not need to conserve heat. The temperature of even the bare hand could be kept within the comfort range at -30°F. When the heat supply to the body was reduced, insulation could not prevent hand and foot temperatures from falling. In two of the three subjects, at -30°F, the extremities rewarmed after the body had been rewarmed. Hand temperature rose before foot temperature. Slight heat deficit caused a fall of foot temperature but not of hand temperature, despite the greater insulation of the foot.

**Effect of epinephrine on the circulating eosinophils.** L. RECANAT (by invitation), P. H. FORSHAM (by invitation), and G. W. THORN, *Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts*

It has previously been shown that epinephrine activates the pituitary-adrenal cortical system by stimulating the secretion of adrenocorticotropin. It was subsequently demonstrated that adrenocorticotropin produced a fall in circulating eosinophils in subjects with normal adrenal cortical function. It has been found that 1.5 mgm of epinephrine in saline given intravenously over a one-hour period produces a fall in circulating eosinophils of from 55 to 75 per cent in four hours in both normal subjects and patients with Addison's disease. The finding that the "adrenalin effect" on eosinophils is independent of the adrenal cortex has been confirmed by the study of normal, adrenalectomized and hypophysectomized rats. The mechanism of action has been studied.

**Arterio-venous blood sugar studies on the kidney of the eviscerated dog.** ROGER M. REINECKE and PAUL J. HAUSER (by invitation). *University of Minnesota*

The concentration of fermentable reducing substance, i.e., blood sugar, usually becomes higher in the renal vein than in the aorta of the dog from

which the liver, pancreas, spleen, stomach and intestine except the lower colon have been removed. This phenomenon is not prevented by stopping the secretion of urine through increasing the pressure in the ureter.

**The effect of changes in cardiac "competence" on right auricular pressure** RICHARD A. RISS (by invitation) and JOSEPH R. DiPALMA *Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.*

Opinion is divided as to the primary factor responsible for the elevated venous pressure encountered in congestive failure. One view relates it to decreased cardiac competence, while the other maintains that it results from the increased blood volume. Experiments in open chest cats in which cardiac competence is decreased by near lethal doses of quinidine intravenously, by electrically induced auricular fibrillation, or by topical application of mecholyl, cause rises in venous pressure (right auricle) of the low magnitude of 1 to 3 cms of water. In competent hearts saline infusions as much as  $\frac{1}{2}$  the total blood volume produce only temporary rises of venous pressure with return to normal values in two to five minutes. When increased blood volume is imposed on an incompetent heart in the same animal, the elevation of venous pressure is still transitory in nature. It is to be concluded that neither cardiac incompetence, increases in blood volume, nor a combination of the two can completely explain the sustained, markedly elevated venous pressures seen in congestive failure. In our experiments to date, prolonged rises in venous pressure were obtained only after the following conditions were produced sequentially: 1) a marked decrease in cardiac competence with quinidine, fibrillation and mecholyl, 2) an increase in blood volume by saline infusion, 3) a partial return of cardiac competence with epinephrine. It is suggested, therefore, that these three conditions, particularly a partial return of cardiac competence, are essential for the persistent elevation of venous pressure.

**Prediction of the stroke volume of man from brachial pressure pulse values** JOHN W. REMINGTON, W. F. HAMILTON, CHARLES R. NOBACK (by invitation), and JAY J. GOLD (by invitation) *Department of Physiology, University of Georgia School of Medicine, Augusta, and Department of Anatomy, Long Island Medical School, Brooklyn*

The volume-pressure relations, corrected for body size, of 48 human aortas, covering an age span from 8 to 89 years, were compiled. The series was divided into age groups, and into a group of hypertensives. *In situ* aortic lengths are the same for all groups. A definite trend for an increase in volume with ageing, and with hypertension, was noted, but the overlap between groups is large. There is no correlation between diastolic size and the vol-

ume uptake per unit pressure increase. Hence the variability of  $\frac{\Delta V}{\sqrt{\Delta P}}$  is considerably greater than that

of the simple ratio  $\frac{\Delta V}{\Delta P}$ . The latter is not significantly changed by ageing nor by hypertension.

Volume distensibility tables were constructed for the calculation of the stroke volume from the pulse contours of 68 intrabrachial pressure pulses, 60 of which were kindly supplied by Dr. André Cournand. Predicted stroke index differs from that given by the direct Fick procedure by an average of 17%. An estimation from pulse pressure alone gives an average discrepancy of 23%. The correction of pulse pressure for the changing arterial distensibility with pressure, lowers the average discrepancy to 18%. The size of the error, and the small improvement made by the use of the contour calculation over the latter procedure, indicate: 1) variation in systolic drainage plays a small part in the inaccuracy of the latter method, 2) that contour variations between brachial pulses is small, and 3) that the major inaccuracy of both methods lies in the variation in arterial distensibility of different individuals.

**Does injury to an axon promptly induce altered excitability in its cell of origin?** BIRDSEY RENSCHAW and HERBERT ROSENBLUM (by invitation) *Department of Physiology, University of Oregon Medical School, Portland*

Two types of experiment on lumbo-sacral motoneurons of pentobarbitalized cats have given no support to the hypothesis that injury to the axon of a neuron promptly induces a change in the excitability of its soma. In one series determinations were made of the size of 2-neuron arc reflex discharges initiated by dorsal root stimulation and recorded electrically in the tibial nerve of the lower leg before and after section of the nerve four to six centimeters peripheral to the assembly of recording electrodes. In a second series micro-electrodes were used to record the tibial nuclear action potentials that were evoked by antidromic motor volleys initiated by delivery of supra-maximal shocks to the tibial nerve in the lower leg before and after section of the nerve below the ankle. Dorsal roots had been sectioned in all experiments. Controls established that section of the tibial nerve did not significantly affect the recording of centrifugal impulses in the first series, nor the size of the antidromic volleys in motor axons in the second. Within four to six minutes after section of the nerve there occurred only very small and inconstant changes in the responses used to measure somatic excitability. If any change is produced promptly as a consequence of nerve section, it is so small under the conditions of the experiments as to be difficult to investigate by the sensitive meth-

ods utilized and would appear to be of doubtful physiological significance

**Are there arterio-venous shunts in the kidney?**

FRANCOIS C REUBI (by invitation), HENRY A SCHROEDER, and ARNOLD H WILLIAMS (by invitation) *Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis, Missouri*

Recently Trueta, et al, using various techniques, demonstrated the probability of arterio-venous by passes in the kidneys of rabbits. Attempts were made to examine this problem in the dog and man. In dogs renal blood flow was measured either by a rotameter in the renal artery or by the renal excretion of para aminohippurate. The extraction ratio of PAH by the kidney was determined as well as simultaneous renal arterio-venous oxygen differences. After the intravenous injection of epinephrine, it was found that the renal arterio-venous oxygen difference decreased almost regularly three to eight minutes later. After ten minutes, it often rose to a higher level than before injection.

In human beings essentially the same methods were employed, renal venous blood being obtained by catheterization. After subcutaneous injection of adrenalin (0.5 mg), the renal A-V oxygen difference decreased markedly a few minutes later in some but not in all cases. The extraction of PAH fell slightly about twenty minutes after injection but this change was not simultaneous with the decrease in the renal A-V O<sub>2</sub> difference. From these results it can be concluded that renal arterio-venous by passes may at times appear under some conditions, but are not of great importance in the total renal circulation.

An analysis of the effects of large volumes of isotonic saline on the circulation of severely hemorrhaged dogs. MONICA REYNOLDS (introduced by MARGUS I GREGERSEN) *Department of Physiology, College of Physicians and Surgeons, Columbia University*

Hemorrhage was produced in 27 dogs by bleeding out and immediately returning 25% of the blood collected (Walcott, 1944). One hour later 0.9% saline was infused slowly (15% of body weight). Changes in the oxygen consumption, arterial and venous oxygen, cardiac output, plasma volume, blood volume, hematocrit and plasma protein were measured. Only four of the animals died. The dogs were thirsty, drank copiously, and excreted large volumes of urine. Plasma proteins and hematocrits shortly after the saline were lower than a day later, the plasma proteins increasing 1 gm %. Eighteen hours after the infusion the plasma volume averaged 8 cc per kgm above the control value but the total blood volume was 15 cc per kgm below the control. Immediately after hemorrhage the cardiac

output was reduced approximately 70%. The saline brought it up to control values and 18 hours later it was 5% above the control. After the bleeding the arterial oxygen concentration fell from 18 to 16 vols %. After the infusion it fell to 9 vols % and the following day it was 10 vols %. The venous oxygen, though lowered from 14 to 4 vols % by the bleeding, rose after the infusion to 6 vols % where it remained. The circulatory changes induced by the saline apparently account for the therapeutic effects observed.

Uterine contractions effective in dilating the human cervix, recorded by the multichannel strain gage tokodynamometer S R M REYNOLDS, L M HELLMAN (by invitation) and P BRAUNS (by invitation) *Carnegie Institution of Washington, Department of Embryology, and Department of Obstetrics, Johns Hopkins University, School of Medicine*

Using a multichannel strain gage tokodynamometer for recording patterns of uterine contractions in gravid women (Science, October 31, 1947), the essential characteristics of contractions which dilate the cervix have been determined. The uterus in the first stage of labor has three functionally distinct regions. The fundus always contracts rhythmically. The lower uterine segment shows no evidence of contraction. The mid-zone of the uterus contracts synchronously with the fundus at the start but it becomes progressively less active as cervical dilatation proceeds. The reason why the mid zone becomes less active is because the contractions here are of progressively less intensity and of shorter duration than in the fundus. Consequently, the work of the fundus, as measured in ergs per unit area is far greater there than in the mid-zone. Deviations from this pattern have been seen in uterine inertia, prolonged labors, and false labor. In multiparous women and in precipitate labors, the fundus may be the only strongly contracting part of the uterus.

Nitrogen balances of dogs continuously infused with 50% glucose and protein preparations. C MARTIN RHODE (by invitation), WILLIAM M PARKINS and HARRY M VARS *Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania, Philadelphia*

Solutions of 50% glucose and glucose protein supplying 45-50 calories/kg/day were constantly introduced into the superior vena cava through an indwelling, plastic, capillary tube and an infusion apparatus which permitted free movement of the dog in a metabolic cage. Equilibration on glucose for 10 days or until urinary nitrogen reaches 100-110 mg/kg/day was followed by 6 day periods on different protein preparations in hypertonic glucose. Calories, nitrogen and fluid volume remained constant throughout. Nitrogen, about 120 mg/kg/day, was administered as casein hydroly-

sate alone, as casein hydrolysate with macromolecular gelatin, in equal amounts, and as gelatin fortified with methionine and tryptophane

Dogs so fed for 4-6 weeks appeared normally active and in good physical condition. The level of urinary nitrogen during these continuous infusions of 50% glucose at 45-50 calories/kg/day was comparable to that reported by others who fed protein-free diets containing 70-80 calories/kg/day by mouth. The nitrogen administered in these experiments was better retained than that injected in single daily doses by others. Average daily nitrogen balances in 4 dogs consecutively receiving glucose, casein hydrolysate as imigen, imigen with Knox P-20 gelatin, and amigen were -106, -15, -26, -6 mg/kg/day respectively. Continuing the study in two of these dogs, comparative values of imigen, fortified gelatin, and amigen were -1, -8, +3. Fifty percent of the intravenous macromolecular gelatin appeared in the urine as precipitable protein.

**An instrument for the measurement of total thermal radiation in the environment.** CHARLES H. RICHARDS (by invitation) and JAMES D. HARBY. *From the New York Hospital and the Department of Physiology, Cornell University Medical College, New York, N. Y.*

An instrument has been designed and built for the purpose of measuring the total thermal radiation under various environmental conditions. For example, in an outdoor environment this measurement is the sum of the direct radiation of the sun, the reflected radiation, the radiation exchange with the sky and with the terrestrial objects. It consists of two hollow spheres of silver, 6 mm. in diameter. Each contains a copper constantan thermocouple soldered to the inside surface and a heating coil of approximately 50 ohms. The balls are supported by stainless steel, #17 hypodermic needle tubing through which are brought out the leads for the thermocouple and heater. Both the balls and the supports are plated with chromium on nickel. One ball is left polished for low emissivity, while the other is blackened with a coating having a high emissivity in the infrared spectral region. In any environment the temperature difference between the black ball and the polished ball will depend on the total radiation exchange between the balls and the environment. Thus, the amount of heat necessary to bring the two balls to the same temperature is a measure of the total thermal radiation. Under these conditions convection effects cancel. Temperature equilibrium reaches 95% in 2 minutes, and relatively rapid changes in the radiation load of the environment can be followed. Typical values of total radiation measurements on a summer day in New York City showed that at 9 AM, before the sunlight was directly incident on the balls, the radiation was minus 60 Cal/hr/m<sup>2</sup>. In direct sun-

light the total radiation increased until at 2:30 PM a measurement of plus 100 Cal/hr/m<sup>2</sup> was obtained. The radiation slowly decreased until shadows fell over the apparatus at about 6 PM and the radiation was minus 15 Cal/hr/m<sup>2</sup>.

**Pulmonary circulation during exercise in normal individuals and in patients with chronic pulmonary disease.** R. L. RILEY (by invitation), A. HIMMELSTEIN (by invitation), H. L. MOELLER (by invitation), H. M. WINTER (by invitation) and A. COLEMAN. *Cardio-Pulmonary Laboratory, Chest Service, Bellevue Hospital, New York City, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.*

Studies of cardiac output and pulmonary arterial pressure were performed using the venous catheter technique in three normal individuals and in eight patients with various types of chronic pulmonary disease. Measurements were made at rest and during exercise on a stationary bicycle. Two of the three normal subjects showed a decrease in the mean pressure in the pulmonary artery during exercise, all showed a marked drop in pulmonary vascular resistance and a minimal increase in the work of the right ventricle during exercise. Three of the patients with chronic pulmonary disease showed a significant elevation of pulmonary arterial pressure at rest, and in all eight cases the mean pressure increased during exercise. There was either no change or an increase in the pulmonary vascular resistance during exercise, and the work of the right ventricle was invariably higher than in the normal subjects at a corresponding work level. The findings indicate that the expansibility of the pulmonary vascular bed during exercise is limited in patients with chronic pulmonary disease. Anoxia may contribute to the elevation of pulmonary arterial pressure during exercise in those patients whose arterial oxygen saturation falls.

**X-ray density changes in the heart recorded by the electrokymograph.** GORDON C. RING, CATHERINE R. MICHIE (by invitation) and M. J. OPPENHEIMER. *Department of Physiology, Temple University School of Medicine.* When an electrokymograph (see G. C. Henny, B. R. Boone and W. E. Chamberlain, *Am. J. of Roent. & Rad. Ther.*, Vol. LVII, 409, 1947) is placed over the middle of the fluoroscopic silhouette of the heart, it will produce curves which look much like cardiometer tracings. One might at first think that these records could be used to measure stroke volume of the heart. This, however, is not possible, since a large heart will have less change in thickness for a given stroke than will a small heart. Assuming that the heart is a sphere and that its total volume (walls plus blood) at the beginning of ejection is 700 cc, the ejection of 200 cc of blood will result in a change in diameter of 1.16 cm, whereas if the starting volume is 500 cc the change for a similar ejection



tion will be 1.54 cm. On this basis, it should be possible if one knows the stroke volume and the amplitude of density changes, to work back and find out how much the heart has dilated.

In testing the above hypothesis, we have produced variations in the stroke of the dog's heart by vagal stimulation and have recorded cardiac density changes, ballistocardiograms and arterial pressures. These results were then plotted as amplitude of density change per cc of output against the stroke or work of the heart. The curves showing small density changes during large change in stroke, we believe, indicate a heart which dilates very little and is therefore in good condition. Epinephrine has a direct effect upon the force of cardiac contraction and when this is given, in spite of the increased arterial pressure, the heart dilates less than during control periods. Our technique makes it possible to demonstrate this in the intact animal.

**Inconsistencies between experimental and theoretical electrocardiography.** JANE SANDS ROBB, *Department of Pharmacology, Syracuse University College of Medicine*

Theory states that the QRS portion of the electrocardiogram reflects the asynchronous excitation of the surface of the heart through the surrounding volume conductor to the indirect leads. In our experiments the ventricles were enclosed in an insulating glass cardiometer, a rubber diaphragm fitted snugly into the a-v groove. The QRS deflections were reduced in amplitude but still apparent. These waves certainly did not reach the indirect electrodes through a volume conductor in contact with the ventricular surface. In other experiments the pulmonary circulation was interrupted and the right ventricular cavity filled with M/5 KCl. Soon afterward, the right ventricle ceased to contract. The electrocardiogram showed S-T shifts and brief QRS deflections in all three standard leads. Internal injury potentials are thought not to affect the indirect leads. Moreover, the theory that the QRS is the algebraic summation of asynchronous, long, oppositely directed, monophasic potentials from the two ventricles would demand a simple monophasic negative wave in this case. If the KCl had depolarized only the endocardial surface, one would expect to see some contraction on the right and a prolonged QRS due to synectial spread through unaffected muscle from the left or to the right ventricle. Finally, when M/5 KCl was placed on the anterior surface or the apex of an exposed heart neither of which had any direct contact with a volume conductor S-T shifts readily occurred. Present theory would demand that only areas in actual contact with volume conductors contribute to the electrocardiogram. Thus, present electrocardiographic theory does not adequately explain many experimental observations.

**The role of the vasa nervorum, especially in regard to "referred pain."** JOSEPH THOMAS ROBERTS, *University of Arkansas School of Medicine, Little Rock, Arkansas, and Gallinger Municipal Hospital, Washington, D C*

The normally abundant blood supply of peripheral nerves was decreased, experimentally and in patients with occlusive vascular diseases. The level and severity of neurological changes were correlated closely with the level and degree of ischemia of the nerves. Ischemic nerves showed histological degeneration. It is concluded that the vasa nervorum play an essential role in maintaining the structure and function of peripheral nerves in a way similar to the role of the blood supply of the central nervous system. The blood supply of the autonomic nervous system seems to play a similar role, according to demonstration of its abundance. These findings are helpful in explaining (1) variation in peripheral neurological complaints, (2) the infrequency of cardiac pain with cardiac hypertrophy and its frequency with other forms of myocardial ischemia, (3) "referred" pain. Somatic nerves of skin, muscle, or body wall may become painful due to vasospastic neural ischemia arising from stimuli with origin in viscera and with a pathway through autonomic nerves attached to the same or adjacent spinal segments.

**Potentiation of the gastric secretory response to histamine by parasympathomimetic drugs.** C R ROBERTSON (by invitation) and M I GROSSMAN, *Dept of Clinical Science, Univ of Illinois College of Medicine, Chicago*

Both histamine and parasympathomimetic drugs stimulate acid secretion by the gastric glands. In the present experiments it has been shown that the response to these two agents acting simultaneously is greater than the sum of their independent actions. Prostigmine methylsulfate (0.5 mg subcutaneously) and urecholine (0.01 mg subcutaneously every ten minutes) produce very little or no acid secretory response in dogs with vagotomized pouches of the entire stomach. However when these drugs are given while the response to histamine dihydrochloride (0.0125 mg subcutaneously every ten minutes) is in progress the rate of acid secretion is markedly increased. In the case of prostigmine the acid production rose from 42.5 mg per hour to 108.3 mg per hour (12 tests on 6 dogs), with urecholine the corresponding figures were 89.9 mg per hour with histamine alone and 221.1 mg per hour with histamine plus urecholine (6 tests on 6 dogs). This potentiation of histamine stimulation by parasympathomimetic drugs has important implications for the understanding of the effect of atropine and the effect of vagotomy on the effectiveness of various gastric secretory stimulants.

**Pretreatment against decompression sickness.** IRLE W ROBINSON (introduced by J W HELM)

*Physiology Branch, Aero Medical Laboratory, Wright Field, Dayton, Ohio*

A series of tests were performed on a group of 21 individuals to determine the best of several pretreatments before prolonged exposure (six hours) to high altitudes. Experiments were carried on both in an altitude chamber at 30,000 and 38,000 feet and in a B-17 airplane at 30,000 feet. At 38,000 feet in the altitude chamber performance was slightly better when the pretreatment consisted in breathing room air while resting rather than in breathing room air while exercising on a bicycle. Noticeable improvement in performance at altitude occurred when subjects breathed oxygen for an hour before ascent. Exercise with oxygen was slightly less beneficial as a pretreatment than rest with oxygen and certainly less popular. At 30,000 feet the same results were obtained. At this altitude exercise was performed in the chamber after ascent. Whereas exercise as pretreatment was slightly detrimental to altitude tolerance, deep knee bends at altitude materially decreased the tolerance. A comparison was made between performance at 30,000 feet in the altitude chamber and in the B-17 airplane. Pretreatment in both cases consisted of breathing oxygen for one hour while resting before reaching 30,000 feet. Deep knee exercises were performed at altitude both in chamber and plane. No significant difference in altitude tolerance occurred. If anything the men did better in the plane than in the chamber, perhaps because they were less bored. Besides indicating a preferable type of pretreatment, these results validate the use of the altitude chamber as a means of determining resistance to decompression sickness under actual flying conditions.

**Further evidence for a temperature-sensitive mechanism in a poikilotherm, the turtle** S. ROXBARD and F. SAMPSON (by invitation). *From the Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Illinois*

We have previously reported evidence for the presence of a temperature sensitive center in cold-blooded animals (Fed Proc., 6:191, 1947). We showed that the relationship which is seen between the body temperature and the arterial pressure of these animals is destroyed by section of the spinal cord or by destruction of the brain. In our present experiments we have warmed or cooled the brain of the turtles by means of a silver wire introduced through a small trephine hole in the skull. Temperature change was produced by heat conduction from a reservoir of water in which the wire was mounted. When the wire was warmer than the brain, the blood pressure, as determined from the aorta by optical recording, increased about 6 to 10 mm Hg. When the wire was cooler than the brain, a fall in pressure of similar magnitude was seen. These results strengthen our belief that a tempera-

ture sensitive center is present in the brain of cold-blooded animals, and that this center functions to adjust the internal environment of the animal to changes in body temperature.

**Sodium and chloride reabsorption** J. C. ROEMMELT (by invitation), O. W. SORRORIUS (by invitation), and R. F. PIRTS. *Dept. of Physiology, Syracuse University College of Medicine, Syracuse, N. Y.*

In studies on normal dogs, preliminary to assessing the effects of adrenal cortical hormones on electrolyte reabsorption, excretion and reabsorption of chloride were determined at plasma levels ranging from 110 to 157 millimols per liter. These levels were attained by infusing 3.5 per cent NaCl at 10 cc per minute. Although in the massed data the reabsorption of chloride per 100 cc of filtrate was constant within limits of  $\pm 10\%$  of the mean, in individual experiments there was a tendency for the quantity reabsorbed to diminish at higher plasma levels. The rates of proximal and distal tubular reabsorption of chloride were calculated from these data according to the formula of H. W. Smith. The values for distal reabsorption were constant within  $\pm 15\%$ . The serum levels for sodium ranged from 135 to 176 millimols per liter. The quantity of sodium reabsorbed per 100 cc of filtrate diminished as the serum level was raised. The distal tubular reabsorption, calculated as for chloride, was constant within  $\pm 15\%$ .

**Oximetric determination of cardiac output in man** ALBERT ROOS (by invitation), J. O. CLAM (by invitation), J. F. NEVILLE, JR. (by invitation) and H. L. WHITE. *Department of Physiology and Laboratory of Applied Thoracic Physiology (Department of Surgery) Washington University School of Medicine, St. Louis.*

Arterial-mixed venous blood oxygen differences have been obtained by a recording modified Millikan oximeter in combination with hemoglobin determinations. Alveolar gas tensions in equilibrium with right heart blood have been produced by one deep inspiration of 6% CO<sub>2</sub> in helium, followed by incomplete expiration to the outside. The breath is then held for several seconds, after which rebreathing of 400 to 500 cc into a bag is permitted. The oximeter curve indicating oxygen saturation falls rapidly, 7 to 10 seconds after onset of fall a plateau, lasting 4 to 10 sec., is reached, which is taken to indicate equilibrium between right heart blood and alveolar air. The plateau is terminated by a second drop, indicating recirculation. During the plateau right heart blood is presumably passing through the lungs unchanged, work to verify this assumption is in progress. Arteriovenous differences, together with oxygen consumption determinations, permit calculation of cardiac outputs, which, in the sitting position, ranged from 2.69 to 2.91 liters per M<sup>2</sup>.

**Relation of basal metabolic rate to vasodilatation and vasoconstriction of extremities of normal subjects as measured by skin temperatures** GRACE M. ROTH and CHARLES SHEARD *Section on Physiology, Division of Physics and Biophysical Research, Mayo Clinic and Mayo Foundation, Rochester, Minnesota*

Maddock and Collier in 1933 first demonstrated a simple linear relationship between the skin temperatures of the great toe and the basal heat production per unit of surface area. In 1940 Sheard and Williams found a similar linear relationship, which seemed to be of a dual character, between the average skin temperatures of the toes and the basal metabolic rate. From a study of 150 observations of normal subjects a linear relationship of dual character was found between the skin temperature of the toes and the basal metabolic rates. The ages of the subjects ranged from nineteen to seventy years and the heat production per unit of surface area ranged from 30 to 50 calories per square meter per hour. After a control period of at least an hour at an environmental temperature of 25.5°C (78°F) when the skin temperatures and basal metabolic rates have been determined, vasoconstriction and vasodilatation of the extremities were produced by placing the subjects first in a cold room at 21°C (69.8°F) for an hour and then in a hot room at 30-31°C (86-88°F) for another hour. It was found that vasoconstriction took place at a more rapid rate and to a greater degree in the subjects with low basal metabolic rates than those with higher rates whereas vasodilatation took place more rapidly in the subjects with higher basal metabolic rates. Basal metabolic rates, therefore, should be considered in studies concerning vasodilatation or vasoconstriction as measured by skin temperatures.

#### **Metabolic balances in the cold environment**

**I. Nitrogen and water exchanges** JAMES L. A. ROTH and JOHN A. FRANTZ (introduced by J. W. HEIN) *Physiology Branch, Aero Medical Laboratory, Wright Field, Dayton, Ohio*

Metabolic observations were made to study the influence of a USAF emergency ration and a restricted water intake upon the nitrogen, water and energy exchanges in human subjects residing continuously for nine days in a cold environment, -32°C. Control studies included fasting for six days in the cold environment and subsistence upon the emergency ration at room temperatures. The design of an emergency ration presents a problem because of the limitation in weight and space allowance in aircraft. Survival for fasting is several times longer than for thirsting. Thus the advantages of a ration must be appraised in terms of its cost to water balance. The ration provided 1948 calories per day, 318 gm carbohydrate, 41.6 gm protein (46 per cent from whole egg) and 56.6 gm fat.

Because of its high biological value, egg protein was used to reduce solute load upon the kidneys.

Body protein sparing contributed by the ration amounted to 70 to 80 per cent of the loss incidental to fasting. No evidence of azotemia, dehydration or hemoconcentration was observed. The greater daily loss of body water during fasting (490 cc compared to 245 cc with the ration) is attributed to a greater release of water from tissue catabolism. The minimum water intake required during the first three days of a fast was slightly less than with the ration, whereas, that required during the subsequent three days of fasting was significantly greater than with the ration. Under the conditions studied, water balance was enhanced during continued ingestion of the ration.

**Production of anaphylaxis in guinea pigs with weakly antigenic protein hydrolysates** L. W. ROTH, R. K. RICHARDS and I. M. SHEPPERD (by invitation) *Department of Pharmacology, Abbott Laboratories, North Chicago, Ill.*

The importance of the total number of sensitizing injections and the duration of incubation period has been established in the course of testing several hundred guinea pigs with partial hydrolysates of protein. When these substances were administered according to the usual techniques described in the literature for strong antigens, doubtful or negative, anaphylactic responses were obtained. If, however, the total number of sensitizing injections was increased, and the period between the last injection and the challenging dose was at least one month, antigenicity could be demonstrated much more regularly. If either factor was diminished, frequency and degree of positive reactions decreased. Doubling or trebling the injection speed favored the production of typical anaphylactic symptoms. Partial despeciation by the hydrolysis was demonstrated by cross shocking experiments. Beef hydrolysate sensitized animals showed anaphylactic reactions when challenged with hog hydrolysate, and vice versa. However, desensitization was not always complete, because in some animals surviving the first shock, the immediate injection of the original sensitizing material produced a second series of anaphylactic symptoms. Reactions were not demonstrable when similar materials were injected into non sensitized animals, ruling out anaphylactoid or peptone like shock. Antigens were not completely precipitable by potassium alum since activity was detectable both in the precipitate and supernatant fluid.

**Rate of penetration of electrolytes into nerve fibers** MORTIMER A. ROTHENBERG (introduced by DAVID NACHMANSOHN) *Department of Neurology, College of Physicians & Surgeons, Columbia University, New York*

The exchange of ions between the nerve fiber and its environment is of paramount importance in the

mechanism of nervous action. Although a few data exist concerning the ion content of the interior of the nerve cell, our knowledge as to the permeability of nerve membranes is limited and based mainly on indirect measurements. The permeability of ions and their rate of penetration have now been measured directly. Giant axon of Squids were exposed to sea water in which the usual ions were replaced by radioactive ones, for varying periods of time. The axoplasm was extruded and the concentration of ions determined with a Geiger counter.—It was found that potassium which is 18 times more concentrated inside than outside, penetrates into the interior against the concentration gradient at a rather high rate. Within 60 minutes, the concentration of radioactive ions was about twice as high in the cell interior as in the outside fluid. Sodium penetrates for the first 20 minutes of exposure at a high rate but then the process virtually stops in spite of the high outside and low inside concentration. At the end of 1 hour, the concentration inside is about one fourth of that outside. Calcium behaves similarly to sodium although the ratio of concentration inside compared to that outside is higher than in the case of sodium. The implication of these findings will be discussed.

**Progesterone or testosterone alter the human vaginal smear** BORIS B RUBENSTEIN *Department of Metabolism Endocrinology Research, Michael Reese Hospital*

There has been controversy whether the ovulation change in the human vaginal smear is due to the presence of progesterone or to the withdrawal of estrogen or combinations of the above hormonal factors. The estrogen induced cornification of the human vaginal smear after castration is altered despite continuous estrogen medication within 24 hours after the injection of 25 mg of testosterone propionate or of 40–80 mg of progesterone. Discontinuing estrogen produces equivalent effects only after 3–5 days. The rapid smear change after progesterone suggests that the previously described "ovulation change" is indeed due to the simultaneous elaboration of both estrogen and progesterone, the latter leading to the rapid desquamation of the cornified epithelium.

**Vitamin E diminishes the vasomotor symptoms of menopause** BORIS B RUBENSTEIN *Dept of Metabolism Endocrinology Research, Michael Reese Hospital, Chicago*

There have been many conflicting reports regarding the value of vitamin E in the treatment of menopause. Some of the discrepancies arise from the fact that 90% of women pass through the menopause without symptoms requiring medical attention, and of the remaining 10%, more than half may obtain adequate relief from small doses of barbiturates, or even from placebos. The present study used as subjects 17 patients with severe vaso-

motor symptoms (hot flashes) following surgical or irradiation menopause. These patients obtained little relief from barbiturates and none from placebos. All obtained prompt and complete relief from Dienestrol (Ortho Products Co.) (0.3–0.5 mg/da). In 14 of the 17 patients, marked reduction of hot flashes (less than 1/da) occurred with 75 mg/da of vitamin E (Ephynol acetate, Roche). Only 6 of the 17 patients obtained complete relief from hot flashes with this medication.

The vaginal smears of all 17 patients showed minimal degrees of cornification after 30 days of medication, equivalent to the cornification produced by 25 mg of testosterone propionate 3X/WK; however, vitamin E produces none of the masculinizing effects of testosterone. Vitamin E may be useful in the treatment of those cases of severe menopausal symptoms in which estrogens are contraindicated.

**A comparison of heat-coagulable serum solids with serum protein determined by a Kjeldahl method** C H WILLIAM RUEL and PAUL L MCLARY (introduced by C C GUTHRIE) *Department of Physiology and Pharmacology, School of Medicine, University of Pittsburgh*

The protein content of serum determined by a micro Kjeldahl method and the heat coagulable chloroform and water insoluble solids determined by the gravimetric method of Guthrie both vary directly with the specific gravity. Since the two measurements are closely related, a comparison of the methods was made on ten samples of beef serum and on ten known dilutions of each sample. The results showed mean values of 50.63 mg protein per ml of serum and 55.19 mg solids per ml of serum. In every case, the value for the solids was higher than the protein value. The difference between the groups was statistically highly significant. Analysis by dilutions to determine whether or not the differences varied with dilution showed that the mean per cent deviation of the solids from the protein varied from 10.32% to 6.95% and averaged 8.61% for all dilutions; there was no apparent relationship between the degree of dilution and the per cent deviation. When the methods were used to determine degree of dilution and applied to known dilutions of serum, they yielded mean errors of 6.1% for the Kjeldahl and 3.4% for the solid method. On this basis and from the standpoint of duplication of results, the solid method was more reliable.

**Sympathetic reaction in peptic ulcer** A H RYAN *Dept of Physiology and Pharmacology, Chicago Medical School, Chicago, Illinois*

The data consists of measurements of a sympathetic response to a standard stimulus. The standard stimulus was a ride of one minute on a bicycle ergometer, and the response, the palmar skin resistance (PSR) measured near the end of the

ride (Ryan and Ranssen, *Am J Physiol* 142 68, 1944) A lower PSR indicates a greater sympathetic response and a higher PSR a lesser response Approximately 500 daily measurements were made on 5 subjects with peptic ulcer (4 were students) over periods of 1.5 to 3 years Two had hemorrhages, all had episodes of pain or other symptoms Forty-five non ulcer subjects (chiefly students), on whom 1360 daily measurements were made, served as controls The mean PSR for the ulcer subjects was 3817, SD1278, and for the controls 2493, SD492 The difference is statistically significant The following table summarizes the average PSR for periods with marked symptoms and periods with few symptoms, for the data available The differ-

| Subject | Marked symptoms | Few symptoms | Difference |
|---------|-----------------|--------------|------------|
| Ca (1)  | 2553            | 2938         | 385        |
| Jo (2)* | 5573            | 5834         | 1261       |
| Br      | 3203            | 3763         | 560        |
| Je      | 2246            | 2751         | 505        |

(1) 3 hemorrhages (2) 2 hemorrhages \* female

ence for each subject is highly significant statistically Evidence of increased sympathetic response generally was present within the two week period preceding symptoms

Some effects of stimulation of the orbital surface of the frontal lobe in the dog and monkey ERNEST SACHS, JR and SAMUEL J BRENDLER (introduced by JOHN F FULTON) *Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn*

Spencer (*Philos Trans*, 1894, B185 609-657) demonstrated that respiratory arrest and blood pressure rise could be obtained by faradic stimulation of the orbital surface of the frontal lobe in cats, dogs, rabbits and monkeys Bailey and Sweet (*J Neurophysiol*, 1940, 3 276-281) have observed similar respiratory arrest and blood pressure rise in the cat and monkey upon stimulation of the posterior part of the orbital surface Delgado, Fulton and Livingston (*Fed Proc*, 1947, 6 95-96) find that certain foci on the orbital surface when stimulated cause an occasional instantaneous fall in blood pressure as well as respiratory arrest (dog and monkey) We have attempted to localize the focus in dogs and monkeys under Dial anesthesia, and our observations are in agreement with the above observers We have obtained respiratory arrest in expiration and we also found it possible to inhibit respiration in any phase of its development On the postero medial portion of the orbital gyrus of the monkey is a circumscribed area of maximal sensitivity for respiratory arrest The threshold for arrest rises upon moving away from this point in any direction even is little as one

millimeter From this same area the blood pressure fall occurred almost immediately without change in pulse Sharp rises in blood pressure were obtained by stimulation of the anterior perforated space in the monkey, using the same intensity and frequency of stimulus as was used for the examination of the orbital gyrus

Extra-thyroidal iodine WILLIAM T SALTER, MACALLISTER W JOHNSON (by invitation), and JAVET BEACH (by invitation) *From the Laboratories of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut*

In hyperthyroid organisms the organically bound iodine in serum, lymph and peripheral tissues is more concentrated than normal In myxedema or marked hypothyroidism the concentration is very low The relative changes, however, vary considerably in different species For example, the skeletal muscle of the normal rat contains only approximately 6 micrograms percent (fresh tissue) as compared with 25 for the cat When extracts of peripheral tissues are fractionated, the organically bound iodine can be separated into arbitrary fractions associated with various protein constituents In the case of skeletal muscle nearly all of the organic iodine is precipitable with the proteins of the tissue, whether the precipitation be made with salt (ammonium sulfate) or with organic solvents The bulk of the organic iodine is precipitated with the muscle globulin ("myosin"), but relatively higher concentrations of iodine are found in the smaller moiety associated with the muscle albumin ("myogen") These observations suggest that the organic iodine is not bound to the protein by simple adsorption, but rather is associated with a specific colloidal carrier

Transverse latency relaxations of muscle stimulated with massive transverse shocks ALEXANDER SANDOW *Washington Square College of Arts and Sciences, New York University* Initially tensed frog sartori, immersed vertically in a curare Ringer bath, are stimulated, isometrically, by transverse massive shocks impressed on two large, plane Ag-AgCl electrodes symmetrically flanking the muscle Transverse mechanical latency changes are recorded by the piezoelectric, cathode ray oscillographic method The pickup stylus is an insulated, vertical, 9 cm long #17 piano wire with a cross bar at its free end which is pressed transversely against the muscle, any point of which may be studied by vertical pickup displacements Due to the great stylus length, latency changes are distorted by the 0.5 ms transmission time along the stylus and an initial slight force at the pickup crystal opposite to that impressed on the stylus end contacting the muscle The second type of distortion is absent for stylus lengths less than 2.5 cm (Thus, Schoepfle and Gilson's observa-

tion, made with a 5 cm stylus, that a water-filled finger cot muscle model transmits a mechanical wave with an initial opposite phase is attributed to a stylus artifact, for it is not obtained with a short stylus.)

The actual experimental records, properly corrected for the stylus distortions, demonstrate that the first response at all muscle points is the latency relaxation, the latency of which is  $1.0 \pm 0.1$  ms throughout the pelvic  $\frac{2}{3}$  portion of the muscle, and about 1.3 ms at the tibial end. Thus the results prove that the latency relaxation is a true physiological precontractile elongation of muscle, and that all portions of at least the pelvic  $\frac{2}{3}$  part are practically simultaneously excited by a transverse shock.

**Observations on the thoracic wall respiratory reflex.** D. SARIS (by invitation), E. A. RILEY (by invitation) and J. C. SCOTT, *Department of Physiology, The Hahnemann Medical College, Philadelphia, Pa.* This investigation differed from the procedure of Whitehead and Draper in using single intravenous injections of pentothal to induce respiratory arrest, oxygen by endotracheal tube, stimulation of the chest wall by application of weights to a freely moving piston, recording of reflex responses by a Marey tambour connected to the tracheal catheter. Thirty-two experiments were performed, mainly on dogs using nembutal anesthesia. With injections of 1 cc of 2.5% pentothal the average recovery rate of excitability of the respiratory center in terms of the strength of stimulus is roughly linear, a maximum stimulus being effective at about  $\frac{1}{2}$  the period of inhibition, a stimulus of  $\frac{1}{3}$  this strength at 75% of the period and a stimulus of  $\frac{1}{4}$  this value at 95% of the total time. Subminimal stimuli produce relaxation of the abdominal wall. The duration of inhibition apparently depends upon the rate of destruction or elimination of pentothal and the rate of accumulation of  $\text{CO}_2$ . Bilateral cervical vagotomy markedly prolongs the period of inhibition and also abolishes the reflex. Section of the right vagus accounts for most of these effects. In one experiment the reflex was elicitable after section of the scalenus medius and rectus abdominis muscles and also by pressure on the parietal pleura in the region of the fourth interspace after the overlying structures had been removed. Present data suggests the reflex may be the Hering-Breuer type.

**Changes in renal function in experimental metabolic acidosis in the normal human subject.** O. W. SARTORIUS (by invitation), J. C. ROEMMELT (by invitation) and R. F. PITTS, *Dept. of Physiology, Syracuse University College of Medicine, Syracuse, N. Y.* It has been repeatedly observed that early in the development of metabolic acidosis increased anion excretion is largely balanced by increased excretion of fixed base. Later,

as the alkali reserve is depleted, increased ammonia excretion permits the conservation of fixed base. The changes in glomerular and tubular function which account for these alterations in fixed base excretion have been studied in a normal subject maintained on constant food and mineral intake for 15 days. From the 6th through the 10th day metabolic acidosis was induced by ingestion of ammonium chloride. Glomerular filtration rate and renal plasma flow were measured in 5 short tests performed at intervals during the control, acidosis, and recovery periods. Daily blood samples analyzed for chloride, sodium, pH, bicarbonate, and phosphate and 24 hour urine specimens analyzed for these same constituents, and in addition titratable acid and ammonia, permitted the description of the renal response in acidosis. The major findings follow. Filtration rate and renal plasma flow decreased during acidosis associated with the reduction in extracellular fluid volume. Excretion of chloride increased in consequence of an increase in the quantity delivered into the glomerular filtrate. In the initial stage of acidosis sodium reabsorption diminished despite reduced filtration, and as a consequence plasma concentration fell. Later with stimulation of ammonia production an increased proportion of the filtered sodium was reabsorbed and depleted reserves were conserved. During the recovery period a maintained high rate of ammonia excretion brought about a rapid restoration of the alkali reserve of the body.

**Development of chronic hypertension in dogs by the occlusion of the carotid arteries.** RONALD E. SCANTLEBURY (introduced by T. L. PATERSON), *Department of Physiology and Pharmacology, University of Arkansas School of Medicine.* In acute experiments blood pressure has been increased by ligation of the carotid arteries. To determine if similar results could be induced in chronic animals a series of experiments was instigated in which the common carotids of dogs were ligated. Blood pressure readings were taken from the hind legs of three dogs by means of a pediatric cuff attached to a Baumanometer. The median of three hourly readings comprised the daily average. Normal blood pressure was determined daily for two months after which the common carotid arteries were occluded with wire sutures. Daily readings were continued during the week recovery period, followed by weekly readings. After operation on April 2nd Dog I, with a normal average systolic pressure of 129, showed a post-operative average of 166 with variations between 138 and 216. Dog II, with a normal average systolic pressure of 132, after operation on June 24th showed an average pressure of 169 with variations between 138 and 195. Dog III, with normal blood pressure of 115, after operation on June 26th showed average pressure of 152 with variations between 148 and

160 Due to the probability of error of the method employed an increase of at least 20 mm Hg was required before the animal was considered hypertensive. The average increase for each of three dogs was 37 mm Hg. These animals were still living on December 6th. Studies to determine tissue changes which might indicate the mechanisms involved have not yet been made.

**Blood electrolyte changes in experimental renal hypertension.** C. A. SCHAFFENBURG (by invitation) and H. SELYE, *Institut de Medecine et de Chirurgie experimentales, Universite de Montreal, Montreal, Canada*. The left kidney was transformed into an "endocrine kidney" in 24 female albino rats (average weight, 150 gm), using a previously described technic [Selye and Stone, *J Urol* 56:399 (1946)]. Post-operatively, they were maintained for ten days on "purina fox chow" and 1% NaCl solution ad lib. Previous observations, made in this Institute, revealed that under these conditions severe renal hypertension ensues. A similar group of unilaterally nephrectomized rats served as controls. After ten days both groups were fasted and given tap water during 24 hours, then they were bled by cutting the jugular vein and the blood was collected under oil in order to obtain serum for biochemical analysis. Seven animals of the first group whose kidneys proved (histologically) to have undergone perfect "endocrine transformation" showed marked hypochloremia ( $85.0 \text{ m eq/L} \pm 6.0$ ) an increased  $\text{CO}_2$  combining power ( $31 \text{ m eq/L} \pm 1.5$ ) and a slightly decreased serum sodium ( $140.7 \text{ m eq/L} \pm 4.9$ ). All these changes were statistically significant but there was no significant change in serum potassium ( $6.12 \text{ m eq/L} \pm 0.16$ ). These findings are of interest especially since we found a similar (although more severe) hypochloremic alkalosis to be accompanied by hypokalemia in rats rendered hypertensive with desoxycorticosterone.

**Electron microscope observations of nerve structure.** FRANCIS O. SCHMITT and EDUARDO DE ROBERTIS (by invitation), *Biology Department, Massachusetts Institute of Technology*. Characteristic fibrous structures have been observed with the electron microscope in macerated preparations of all types of nerves thus far examined, vertebrate and invertebrate. Most observations were made on formalin fixed material though the structures were observed also in macerated fresh frog sciatics and spinal roots. They were also obtained from fixed squid giant axons freed of non axonic material. The chief features are the dense edges staining intensely with phosphotungstic acid, a core of relatively low density and an axial periodicity in the form of transverse bands. In human sympathetics the average width is about 600 Å and the axial period is about 650 Å, three intra period bands of characteristic density and

position have been observed. A dense amorphous material associated with the structures laterally is removable by water extraction.

The available evidence is consistent with this view that the fibrous axonic structures may be cylindrical or tubular in nature and are of indefinite length (at least many microns). They disintegrate very rapidly in macerated fresh material. In excised bull frog spinal roots kept in the cold the structures remain intact until the irritability is lost (8 to 10 days). Thereafter mainly disintegration products are observed. For these experiments action potential data were obtained, nerves fixed in formalin after various periods of survival and macerated preparations examined. Other details of structure of nerve fibers, as observed in the electron microscope, will be discussed.

**Measurement of electrical energy release, "impedance", and longitudinal transport in nerve by differential electrode techniques.** OTTO H. SCHMITT, *Departments of Zoology and Physics, University of Minnesota, Minneapolis, Minnesota*. By using closely spaced electrodes and high amplification instead of conventional nerve electrodes widely spaced or referred to a killed point, it is possible to measure directly the instantaneous axis cylinder current in single nerve fibers. Starting from this information and making free use of the new electronic techniques for differentiation and integration, it is easy to obtain directly on an oscillograph trace, measures of the electrical energy released at the nerve membrane during activity of the generalized "Impedance"-source function which the membrane must represent, of the instantaneous charge deposited at the membrane, of membrane current, and even of the monophasic action potential. Introduction of the new concept of a generalized impedance source complex to replace the separate conventional concepts of a polarizing "battery" and an A.C. impedance characteristic will be justified. Of special interest is the direct theoretical correlation found between the nerve after potential distribution and the longitudinal movement of charge bearing material in the axon.

**Nerve excitation as a function of membrane voltage.** GORDON M. SCHOEFFLE, *Dept of Physiology, Washington University School of Medicine, St. Louis, Mo*. Excitation of frog nerve was investigated in relation to voltage changes on the surface of the nerve induced by threshold rectangular current stimuli of various durations. It is assumed that voltage across the membrane in the extrapolar region is proportional to the voltage of the external fluid in accordance with the cable theory of core conduction. For various durations of threshold current, curves of peak voltage as a function of extrapolar distance along the nerve surface were found to intersect at a locus corresponding to a region about

7 mm distant from the cathode. In view of the very low magnitude of potential observed at such a great distance from the cathode it is concluded that these findings do not offer a direct measurement of the minimal length of nerve which must be raised to a critical level of excitation prior to initiation of a propagated response. In describing the time course of excitability at regions adjacent to the cathode a solution of the equation

$$\frac{d\lambda}{dt} = C(y)(P_0 - \lambda) - k(\lambda)$$

is found applicable as a first approximation. For this equation  $y$  is considered to be the concentration of a cation accumulated across the membrane capacity in direct proportion to the membrane voltage, and  $\lambda$  is the fraction of a membrane constituent of original concentration  $P_0$  which combines with  $\lambda$  to form a complex which when present in sufficient concentration produces instability of some membrane component and hence leads to response of the nerve.

**Pressor substances in hypertensive blood**  
HENRY A. SCHROEDER, MELVIN L. GOLDMAN (by invitation) and NORMAN S. OLSLA (by invitation). *Departments of Internal Medicine and Biological Chemistry and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri.* Arterial blood from hypertensive and normal subjects was extracted according to the method of Schroeder and Stock (J Clin Invest 21:627, 1942). Blood extracts were further purified by a method developed by one of us (N. S. O.). Blood pressure in the rat was measured directly by a Hamilton manometer, and the effect of the intravenous injection of blood extracts was determined. The crude extracts contained substances producing three effects, i.e. depressor, pressor, and one resulting in bradycardia. Purified extracts contained only depressor or pressor substances. The latter were not observed in extracts taken from the blood of normotensive subjects. Most hypertensive individuals contained in their blood pressor material which also gave a VEM reaction in the vessels of the rat's mesoappendix. Further attempts to isolate, purify and characterize this substance will be discussed.

**Relation between growth and synthesis of lipid in colpidium** GERALD R. SEAMAN (introduced by CHARLES G. WILBER). *Biological Laboratory, Fordham University.* *Colpidium capylum* was grown in sterile, fat free proteose peptone solution and the amounts of lipids produced were estimated by standard biochemical procedures. The results were correlated with the growth phase of the respective cultures. Cholesterol was not found at any time in the cultures or in the colpidia. Table 1 shows the amounts of fatty acids present in the organisms in relation to the population of the cultures. Lipid phosphorus remains at a constant

value of 0003-0005 mg/1000 organisms. During the logarithmic phase of growth the amount of lipid phosphorus in the culture fluid remains at a constant level of 6.1-6.8 mg/100 cc. The fatty acids in the culture fluid at the beginning of the logarithmic phase decrease in amount from 21.5 mg/100 cc to 13.9-15.2 mg/100 cc and remain at that low level until the equilibrium phase of growth is reached.

| Age of culture | Populations in 1000 organisms | Mg fatty acids/1000 organisms |
|----------------|-------------------------------|-------------------------------|
| days           |                               |                               |
| 2              | 13.2                          | 0022                          |
| 3              | 23.1                          | 0007                          |
| 4              | 57.3                          | 0020                          |
| 5              | 61.6                          | 0014                          |
| 6              | 62.9                          | 0010                          |
| 7              | 59.5                          | 0016                          |
| 8              | 33.5                          | 0012                          |

The amounts of lipid phosphorus and of fatty acids in the culture fluid increase at the beginning of the equilibrium phase and continue to increase during the declining phase of growth of the culture.

**Fetal gastric secretion of radioiodine applied precutaneously to pregnant animals** W. A. SELLE and OTIS B. MILLER (by invitation). *Departments of Physiology and Dermatology, University of Texas, Medical School, Galveston.* Iodine 131, in ointment or aqueous solution, was applied to the denuded, unbraised skin of pregnant rabbits and guinea pigs near term over an area of approximately 20 sq. cm. Following intervals of 12 to 24 hours the animals were sacrificed and the fetuses removed from the uterus with care to prevent contamination by the mother's skin, blood, urine. Samples of the amniotic fluid, urine, blood and of most of the organs were taken for examination by Geiger Counter techniques. The distribution of radioiodine in the fetus was found to be quite similar to that of adult animals. While the fetal thyroid took up large amounts of the precutaneously transmitted iodine, more than 90% of the total fetal iodine was located in the gastric contents. The lung, kidney, and urine contained relatively high percentages, whereas the blood, liver, spleen, bile and submaxillary gland contained intermediate amounts. Amniotic fluid, muscles, skin, and intestine contained low percentages. The mechanisms of secretion and concentration of iodine by the stomach were not determined. It is thought that the iodine is secreted in a manner similar to that of chloride.

**Further studies concerning brain lesions induced by desoxycorticosterone overdosage in the rat** HANS SELYE, PIERRE HAOUR (by invitation) and CLAUDE FARIBAUT, (by invitation). *Institut de Medecine et de Chirurgie Experimentales, Universite de Montreal, Montreal, Canada.* Previous investigations have shown that in addition to nephrosclerosis, hypertension and periarteritis nodosa,



overdosage with desoxycorticosterone acetate (DCA) can produce severe morphologic changes in the brain (Selye et al. *Exper Med and Surg* 2, 224, (1944), Selye, *J Clin Endo* 6 117 (1946)) In continuation of this work 20 young rats (72 gm) were given subcutaneous implants of three 20 mg DCA pellets following sensitization to the toxic effects of the latter by unilateral nephrectomy. All animals were kept on "purina fox chow," but received 1% NaCl, instead of tap water, as a drinking fluid. During the course of the experiment it was noted that several of the animals showed marked neurological manifestations such as convulsions, motor disturbances in the fore limbs or signs of general excitement and confusion. They also revealed a typical skull deformity, characterized especially by the enlargement and rounding of the calvarium, similar to that seen in the spontaneous hydrocephalus of the rat. After 43 days of treatment, at autopsy, these animals exhibited an increased average brain weight, multiple petechial hemorrhages under the surface of the hemispheres, an increased amount of cerebrospinal fluid and certain histologic changes in the nervous tissue which will be described in detail.

**Measurement of red-cell volume loss by the use of methemoglobin-tagged cells.** O W SHADLE and JAMES C MOORE (introduced by HAMPDEN C LAWSON) *The Dept of Physiology, Univ of Louisville School of Medicine*. The total circulating red cell volume was estimated in barbitalized, splenectomized dogs before and after hemorrhage by the methemoglobin tagged cell method of Moore and Shadle (*Fed Proc* 7 S2, 1948). When arterial pressure was maintained by injecting gelatin solutions after the hemorrhage, good agreement was obtained between the first circulating cell volume and the sum of the post hemorrhage volume and the volume of cells drawn. In a series of six dogs, the agreement between these two values was always within 4 per cent, with random directions of disagreement. In five additional experiments, mean arterial pressure was stabilized between 30 and 50 mm Hg, after the hemorrhage, by additional bleedings or re injections, before making the second determination. The post hemorrhage volume, plus the cell volume drawn, was significantly less than the first circulating volume in only one dog, which suggests trapping of cells at these levels of arterial pressure. In the remaining animals, although the reduction in cell volume measured by the second determination was always slightly excessive, deviation from the expected values did not exceed the variations observed when arterial pressure was maintained.

The renal clearance of some polyethylene glycols in the dog. C BOYD SHAFFER, FRANCES H CRITCHFIELD and CHARLES P CARPENTER (introduced by J M ROGOFF) *Chemical Hygiene Fellowship Mellon Institute, Pittsburgh, Pennsylvania*

The polyethylene glycols (PEG) are a series of unctuous, water soluble compounds varying in consistency from viscous liquids to pasty or solid materials. Structurally, they are characterized by a large number of ether linkages together with terminal hydroxyl groups, and they possess a low order of chemical reactivity. In this study the renal clearance rate of several PEG ranging in average molecular weight from 400 to 6000 have been investigated in the dog using creatinine as the reference substance. Determinations of PEG were made by a gravimetric method (Shaffer and Critchfield, *Anal Chem*, 19 32, 1947).

Experiments were performed on female dogs lightly anesthetized with pentobarbital. The PEG was administered in saline solution in concentrations up to 5 per cent by constant intravenous infusion at a rate of about 2-4 ml/min. A total of 59 periods were obtained in 16 experiments on PEG of average molecular weights of 400, 1000, 1540, and 4000. Taking these 59 periods as a group, the average ratio of the PEG clearance to the simultaneous creatinine clearance was 0.99 with a standard deviation of 0.06.

The ratio of the clearance of a PEG of average molecular weight 6000 to that of creatinine was consistently and significantly less than 1. This circumstance was essentially unaffected by extreme hyperglycemia and by the intravenous infusion of diodrast in an amount sufficient to saturate tubular excretory activity.

Injected intravenously, the PEG are distributed in a fluid volume approximating that of the extracellular fluid of the body.

**Medium concentration in relation to the water content and electrical properties of nerve.** ABRAHAM M SHANES *Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D C, Marine Biological Laboratory, Woods Hole, Mass, and Bermuda Biological Station*. Rana frog sciatics decrease in weight to  $85 \pm 1.5\%$  in Ringer made 2X hypertonic by the addition of either KCl or NaCl, sucrose is significantly more effective, equivalent amounts (according to freezing point data) reducing the weight to  $78.6 \pm 0.62\%$ . Hypertonicity with sucrose decreases the resting potential but increases the action potential, while excess NaCl, applied to the entire trunk, reduces the action potential reversibly. Weight changes were completely reversible after ca. 20 hours in hypertonic solutions. *Libinia* Spider crab leg nerves are freely permeable to KCl. Thus, the same degree of weight increase results whether distilled water or "isotonic" KCl is used to dilute the artificial sea water medium. Sucrose, glucose, and NaCl are less effective, in the order given, in causing weight loss when added in equivalent amounts to sea water to make a 1.5X hypertonic medium. The addition of KCl to the medium

has no prolonged, effect on the weight, although in other invertebrate nerves a reversible increase occurs. Hypertonic solutions decrease the resting potential, but conduction of the motor fibers is not prevented even in 2X solutions of NaCl. *Loligo*. Tonicity changes of the order 25-50% render the squid giant axon irreversibly inexcitable. Microscopic observation suggests that the sheath limits swelling in hypotonic sea water, in very hypertonic solutions (5X) the cytoplasm pulls away from the sheath. No osmotic change occurred in isotonic KCl for as long as ca. 15 minutes, and excitability was restored upon return to sea water.

**The alteration in osmotically inactive fraction produced by cell activation.** HERBERT SHAPIRO *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland*. Resting and activated eggs of the sea urchin, *Arbacia punctulata*, were swollen in hypotonic sea water (60, 70, 80, and 90 per cent), and allowed to attain equilibrium, after which volumes were determined from filar ocular micrometer measurements of their diameters. Both fertilized and unfertilized eggs obey the Boyle-van't Hoff law ( $P(V - b) = RT$ ), but the value for "b", the osmotically inactive fraction ( $\phi_i$ ), or non-swellable volume was different for the two, averaging in the cases studied, 7.3 per cent for unfertilized, and 27.4 per cent for fertilized. On activation in normal sea water, the eggs of the sea urchin undergo a definite increase in total cell volume of approximately 4 per cent. Evidence is available that the alteration in  $\phi_i$  and in cell volume may depend upon the species in question. In two species a parallelism between change in "b", and alteration of respiratory metabolism on fertilization was found, though this may not prove to be a general rule. Equations for the calculation of the point at which osmotic pressures and cell volumes are identical for unfertilized and fertilized are included. A mechanical analogue of the phenomena is introduced.

**The retarding action of vitamin C in physiological concentrations on the rate of cell division.** HERBERT SHAPIRO *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland*. A study of the pathological changes in ascorbic acid deficiency indicates that the primary effect is an abnormality in the intercellular material or perhaps an effect at the cell surface, resulting in changes in the bones and small blood vessels. The phenomena are of cellular physiological interest, as they point to some relation between cell structure and pathology, arising from a biochemical deficiency. Ascorbic acid apparently plays no major role in tissue respiration, hence it appeared promising to investigate the influence of l ascorbic acid on cell division, as possible evidence of action on the cell surface. Fertilized eggs of the sea urchin, *Arbacia punctulata*, were exposed at constant temperature to con-

centrations of vitamin C in sea water, varying from  $2.62 \times 10^{-6}$  molar to  $1.57 \times 10^{-3}$  molar. The rate of cell division was determined by measuring the time required for 50 per cent of the cells to pass through first cleavage. Beginning with approximately  $10^{-5}$  molar solutions, a definite retardation of cell division was observed. At high concentrations, complete inhibition of division occurred. The slowing down of the rate of cell division appeared to be roughly a linear function of the concentration, beyond a minimal effective concentration.

**The effect of "carbonation" on the rate of gastric emptying and intestinal absorption of sucrose solution.** K. L. SHAPIRO (by invitation) and A. C. ILLI *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago*. Ten healthy adult male subjects between 24 and 32 years of age, were each fed after a 12 hour fast, 200 cc. of an uncarbonated solution of 10% sucrose in distilled water. The fluid volume and sugar content of this amount of solution are approximately equal to those of the average carbonated "soft drink" beverage widely consumed to day. Venous blood samples for quantitative sugar determinations were drawn just prior to the administration of the solutions and at intervals of  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2, and 3 hours after its ingestion.

On another occasion, under the same conditions, and after a similar 12 hour period of fasting, the same ten subjects were each fed 200 cc. of a carbonated solution of 10% sucrose in distilled water. Venous blood samples for blood sugar estimation were drawn at intervals equal to those of the previous period of observation. The carbonation had no significant effect.

**Effect of phytate and other food ingredients on the absorption of radioactive iron.** LEON M. SHARPE (by invitation), ROBERT S. HARRIS, WENDELL C. PEACOCK (by invitation), and RICHARD C. COOKE (by invitation) *Departments of Food Technology and Physics, The Massachusetts Institute of Technology and the Walter E. Fernald State School*. Fifteen boys, 12 to 17 years old, living in an institution, were fed breakfasts of the following test meals: I, 200 ml. water, II, 200 ml. milk, III, 200 ml. milk plus 234 mg. phytic acid as the sodium salt, IV, 200 ml. milk, plus 234 mg. phytic acid as in rolled oats (285 gms.), V, 200 ml. milk, plus 285 gms. rolled oats, 150 ml. tomato juice, 75 gms. egg omelet and 56 gm. white bread. Total iron content of all meals was equalized with V at 8.31 mg. using  $\text{FeCl}_2$ . Ascorbic acid (32 mg.) added to reduce all iron to ferrous form. I and II contained no phytate.  $\text{Fe}^{65}$  and  $\text{Fe}^{59}$  were used alternately. No food was eaten before or until five hours after the test meals. Radioactive iron absorption was calculated from activity of hemoglobin, plasma volume and hematocrits. Maximum radioactivity of the hemoglobin was reached within 10 days after feeding. Average percentage of ingested iron appearing in the hemo-

globin was I, 12.4, II, 7.7, III, 0.8, IV, 4.8 and V, 2.5 Sodium phytate markedly affected iron absorption and is five times more reactive than that in oatmeal. Milk and milk plus oatmeal decreased iron absorption by  $\frac{1}{3}$ rd and  $\frac{2}{3}$ rd, respectively. Thus phytate free milk interfered with iron absorption nearly as much as phytate rich oatmeal.

**Adenosinetriphosphatase and conduction of the nerve impulse.** WILHE SHARPLES (by invitation), HARRY GRUNDFEST and DAVID NACHMANSON, *Dept of Neurology, College of Physicians & Surgeons, Columbia University, New York.* Adenosinetriphosphatase has been found in high concentrations in the giant nerve fiber of *Loligo pealii* after the major part of the axoplasm has been extruded (Libet, *Biol Bull*, 93: 219, 1947). On the basis of this finding, that adenosinetriphosphatase like cholinesterase is concentrated in the surface of the fiber, Libet suggested that permeability changes of the nerve membrane associated with electrical activity may in turn be closely associated with adenosinetriphosphatase activity. This hypothesis has been tested by *in vivo* and *in vitro* experiments on frog sciatic nerves, by the use of anticholinesterases that abolish electrical activity rapidly. Eserine abolishes conducted electrical activity of nerve reversibly. It does not affect adenosinetriphosphatase activity even in  $10^{-3}$  M concentration whereas cholinesterase activity is abolished in concentrations of  $10^{-5}$  to  $10^{-6}$  M. Similarly, diisopropyl fluorophosphate which abolishes the electrical activity reversibly or irreversibly depending upon controlled experimental conditions, does not affect activity of adenosinetriphosphatase at a concentration of  $10^{-3}$  M whereas it abolishes cholinesterase activity in concentrations of  $10^{-5}$  to  $10^{-6}$  M.

**On the use of venous pressure in the head as a tolerance index of negative "G" in humans.** R. S. SHAW (by invitation), J. L. GAMBLE, JR. (by invitation), P. J. MAHER (by invitation), J. P. HENRI and O. GAULR (by invitation), *Aero Medical Lab., Air Materiel Command, Wright Field, Dayton, Ohio.* Injury to animals from negative acceleration of over three seconds' duration first appears as rupture of small vessels about the head. Consequently, it was thought that venous pressure in the head might be used as an index of tolerance in humans exposed to negative acceleration on the centrifuge. Four subjects were secured in a seat with an adjustable back mounted on the centrifuge, and venous pressures during negative acceleration were measured with a variable inductance manometer. Venous pressure in the frontal vein of human subjects exposed to negative acceleration was found to vary linearly with the magnitude of the acceleration as though from a simple hydrostatic column based at the heart; they ranged from 80 to 110 mm Hg. No appreciable movement of the heart was noted in X rays of the chest taken under negative accelera-

tions of 3 g. "Red-out" was not observed in this series. Pressures were recorded with subjects in 45 degree and 60-degree backward tilted seats, and compared with those obtained in the standard 10-degree seat tilt. No significant protection against negative acceleration as indicated by venous pressures in the frontal vein resulted from this tilting. Breath holding and straining maneuvers, which increase venous pressure in the resting subject, enhanced the disagreeable subjective sensations, while closing the glottis and attempting inspiration (Müller Maneuver), which decreases venous pressure, resulted in considerable alleviation of symptoms under negative accelerations up to 3 g.

**Courses of dark adaptation and levels of vitamin A and carotene in normal and clinical conditions.** CHARLES SHEARD, *Division of Physics and Biophysical Research, Mayo Foundation and Mayo Clinic, Rochester, Minnesota.* This presentation is concerned with tests on the levels and courses of dark adaptation of rods and cones, on the one hand, and, on the other hand, the levels of vitamin A in the blood serum in a group of seventy persons clinically selected because of a history or symptoms indicative of possible avitaminosis A. Standards, criteria and controls used in the determination of dark adaptation levels are those established by the author. Standards regarding high, average and low values of vitamin A (I U) and carotene (I U) per 100 cc of blood serum are those established by Osterberg and his associates. In 60 per cent of the group tested, data show high threshold levels and delayed recovery of both rod and cone dark adaptation with very low fasting levels of carotene and vitamin A and with levels of vitamin A less than 500 I U per 100 cc of blood serum six hours after ingestion of 7,500 units of vitamin A per kilogram of body weight. From the data the following conclusions are drawn: 1. The courses and the levels of dark adaptation are not in agreement with the normal (standard) findings when the fasting levels of carotene and vitamin A are very low and are less than 100 I U and 150 to 200 I U per 100 cc of blood serum respectively, or, again, less than 500 I U per 100 cc of serum six hours after the ingestion of 7,500 units of vitamin A per kilogram of body weight. 2. In some cases the levels of dark adaptation are elevated 0.5 to 1.0 log unit for the cones and as much as 1.5 log unit for the rods. 3. Definite cone knees (plateaus) are exhibited in the courses of dark adaptation in peripheral areas; these effects persist for as much as ten minutes and are produced under conditions of previous light adaptation which would not cause such results in persons with normal dark adaptation and high vitamin A and carotene levels in blood serums. 4. In perimetric surveys made by the technique of dark adaptation used by the author, it has been possible to show marked reductions in the functioning of the rods in certain areas

and, in some instances, marked improvement therein subsequent to vitamin A therapy or other medical treatment

**Inhibition of sweating by means of iontophoresis** WALTER B. SHILLER, PLATER N. HORVATH (by invitation) and SILVIA M. HORVATH *Departments of Dermatology and Physical Medicine, University of Pennsylvania School of Medicine* After treatment of areas of normal human skin with iontophoresis, in which distilled water, methylene blue solutions, and other compounds were used under the positive electrode, sweating was found to be inhibited. The skin appeared normal when the subject was not sweating but when profuse sweating was induced, multiple vesicles appeared at the sites of sweat gland openings. The inhibition of sweating was present immediately after treatment in some subjects, but the vesicles could not be made to appear until after a variable latent period. The effect was not permanent since normal function of the sweat glands returned after one to three weeks.

**The permeability of normal human skin to histamine** WALTER B. SHILLER and FRANK M. MELTON (by invitation) *Department of Dermatology, University of Pennsylvania School of Medicine* A study has been made of certain of the factors influencing the penetration of histamine through normal intact human skin. The skin was found to be permeable to histamine both in the form of the salt and the free base. However, histamine free base penetrated the skin more readily than the histamine diphosphate. A second important factor was found to be the concentration of histamine which dissolved in the vehicle. Uniformly, as the concentration of histamine in solution increased, the magnitude and duration of effect increased. Solutions of 0.1% strength had no effect on the skin where a 1% solution (calculated as free base) produced local erythema, urticaria and pruritus. Water proved to be among the more effective of 21 semisolid and liquid vehicles studied. In the case of liquid vehicles, covering the injection site greatly prolonged and increased absorption by preventing evaporation. Individual differences in the permeability of the skin to histamine were striking but in all subjects the application of a 10% aqueous solution of histamine free base produced marked local erythema, urticaria and pruritus.

The failure of certain previous investigators to demonstrate the permeability of the skin to histamine appears to stem from the fact that the histamine salt was used in low concentrations and in vehicles which did not promote absorption.

**The composition of polysaccharides of human cervical mucus** LANDRUM B. SHILLER and ZACHARIAS DISCHE (by invitation) *Departments of Obstetrics and Gynecology, Anatomy and Biochemistry, College of Physicians and Surgeons, Columbia University* The presence of substances reducing

after hydrolysis in human cervical mucus was reported by Viergiver and Pommerenke and of glucosamine in bovine cervical mucus by Boyland. We investigated the nature of polysaccharides in the translucent, acellular, mideclic human cervical mucus with the cystin reaction of carbohydrates described by Dische, which enables one to detect individual hexoses and various classes of true sugars in mixtures and which was adapted by us for quantitative purposes. The total amount of true sugars in the mucus ascertained by the indole method of Dische and Popper varied between 0.120 and 0.250%. Up to 20% of it consisted of methylpentose and up to 55% of galactose. The rest was glucose. No significant amounts of hexoketoses, mannose or hexuronic acids were found. The ratio galactose/methylpentose varies between 2.5 and 2.9. This ratio appears identical with that found with the same and other methods in various preparations of blood group substances isolated by Kabat and collaborators from linings of stomach mucosa of hogs. The methylpentose and galactose of the mucus are precipitated by 90% ethanol. These findings suggest that the main bulk of polysaccharides of human mideclic cervical mucus consists of a substance identical with or closely related to one of the blood group substances.

**Observations on the sustained pressor principle in different animal species** ROBERT E. SHIPLEY and O. M. HELMER *Lilly Laboratory for Clinical Research, Indianapolis General Hospital* Blood plasma obtained from dogs and rats which had been subjected to  $\frac{1}{2}$ -2 hours of hypotension (40-50 mm Hg) possessed the ability to cause a sustained elevation of blood pressure when injected intravenously into dogs and rats which had been bilaterally nephrectomized 1-3 days before. Plasmas from normal dogs or rats did not cause sustained pressor responses. These findings parallel the observations originally made on cats (Shipley et al., *Am J Physiol* 119:708, 1947).

Semi-crude extracts of cat, dog, sheep, and hog kidneys were injected intravenously into cats and dogs and the animals bled 1 hour thereafter. The plasmas so obtained possessed the ability to cause sustained pressor responses in nephrectomized cats, dogs, and rats.

**Experimental displacement of the acid base equilibrium of the blood in aged males** NATHAN W. SHOCK and MARVIN J. YIENGST (by invitation) *Division of Physiology, National Institute of Health, Bethesda, Maryland, and Baltimore City Hospitals, Baltimore, Maryland* Experimental displacement in the acid base equilibrium of the blood were induced in six males, aged 70 to 90 years by oral administration of sodium bicarbonate or ammonium chloride. Blood samples were drawn at  $\frac{1}{2}$  to 1 hour intervals for 9 hours after administration of the salt, and analyses for  $\text{pH}_a$ ,  $\text{V}_a$ , total  $\text{CO}_2$  con-

tent,  $p\text{CO}_2$  and bicarbonate content of the plasma were made by the Shock-Hastings micro method, blood samples were also drawn at 24, 28, 32, 48, 52, and 56 hours when necessary to obtain normal values. Results on the aged were compared with those previously reported on young adults (Shock, N W and Hastings, A B, J Biol Chem, 112: 239-252, 1935). Acid base paths of displacement and recovery and rates of recovery of plasma bicarbonate content were plotted for each experiment. Preliminary results indicate that recovery from metabolic acidosis induced by the oral administration of ammonium chloride is significantly slower in the aged subjects tested than in the young adults. Eight hours after the administration of 10 gms of ammonium chloride, the average recovery of blood bicarbonate levels was 70 per cent complete in the young subjects, while in the old subjects the average recovery was only 27 per cent complete. The rate of elimination of orally ingested sodium bicarbonate was only slightly slower in aged males than in young adults. It was also observed that the respiratory compensation for a metabolic alkalosis observed in young adults did not occur in the aged subjects when 20 grams of sodium bicarbonate were administered orally.

#### Hepato-renal factors in circulatory homeostasis

**XV Vasotropic content of blood in chronic hypertension (dogs, man)** EPHRAIM SHORR, B W ZWEIFACH and S BALZ (by invitation) *Dept of Medicine, Cornell University Medical College and The New York Hospital, New York City*. During the development of renal hypertension (Goldblatt clamp) in the dog, blood samples, assayed by the rat mesoappendix test, produced a vaso excitator effect on the terminal arterioles and precapillaries. Later, with the development of chronic hypertension little or no VEM could be detected in the blood stream by this method. This "neutral" reaction was found to represent a counterbalancing of the augmented VEM titer by a comparable increase in the oppositely acting VDM principle. This was demonstrated by aerobic incubation of "neutral" blood samples with normal kidney slices *in vitro*, a procedure which results in the inactivation of the VEM and thereby reveals the absolute concentration of VDM in the blood. Blood samples taken from patients with chronic essential hypertension and adequate renal excretory function were likewise found to produce a "neutral" effect in the test rat. The question arose as to whether the "neutral" effect in the blood of man represented a similar state of equilibrium with high titers of both VDM and VEM. This was conclusively demonstrated in a series of 11 essential hypertensives in which the blood was similarly incubated with normal kidney slices and tested for VDM activity. All of the blood samples from hypertensive patients produced pronounced VDM effects following *in vitro* aerobic in-

cubation. A series of normotensive hospital patients on a similar diet gave essentially neutral effects in the rat test both before and after *in vitro* incubation. These observations provide another point of similarity between essential hypertension in man and experimental renal hypertension.

**Hepato-renal factors in circulatory homeostasis XIX VEM and VDM mechanisms in nutritional cirrhosis in rats** EPHRAIM SHORR and B W ZWEIFACH *Dept of Medicine, Cornell University Medical College and The New York Hospital, New York City*. Rats on a low protein (10% casein) diet supplemented with cystine develop cirrhotic like changes in liver and kidney (Gyorgy). The poor tolerance of these rats to surgical procedures suggested that this predisposition to shock might be related to disturbances in the renal VEM and hepatic VDM mechanisms. Cirrhotic rats prepared according to Gyorgy were studied after 120-150 days on a protein deficient diet. Saline washes of liver invariably contained VDM. There was no impairment of the ability of the liver to form VDM anaerobically *in vitro*. The significant hepatic lesion was the failure to inactivate VDM aerobically *in vitro*. The kidneys were also found to have an impairment of their VEM mechanism, little or no VEM being produced on anaerobic incubation *in vitro*. Blood from cirrhotic rats produced in normal test rats a moderate vaso-depressor response in the terminal mesenteric arterioles and precapillaries, indicative of the presence of VDM. The mesenteric blood vessels of cirrhotic rats were hypo-responsive to the topical application of epinephrine. These phenomena are considered to reflect the derangements of the VEM-VDM mechanisms in the cirrhotic rat, as a result of which the vascular bed is chronically under the decompensatory influence of VDM. These derangements in the VDM-VEM mechanisms, because of their significance for compensatory vascular reactions to shock, are believed to contribute to the predisposition of the cirrhotic rat to shock.

**The leakage of potassium from injured muscle fibres** F J SICHEL and CHERYL PARKHURST (by invitation) *University of Vermont College of Medicine*. Previously the rate of potassium loss from transversely cut frog skeletal muscle fibres into a restricted volume of Ringer's solution was reported. The rate was sufficient to account for conduction failure within a few minutes. Since this failure involves the entire length of each fibre in such a short time, the possibility must be considered that local trauma might change the entire surface of the fibre so that potassium loss occurs through this surface and not only through the cut ends. The surface shows no visible changes except where cut, although permeability might be altered. If the potassium came out mostly through the uncut surfaces there should be very little difference in

the rate of potassium loss in fibres cut once and cut three times. If the loss were mostly through the cut ends, there should be a greater rate of loss with more cut ends.

The muscles, in a restricted volume of Ringer's solution, were cut transversely either once or three times. The rate of accumulation of potassium in the medium was determined by means of a flame photometer. It was found that the rate of leakage of potassium was much higher in the case of those muscles with three transverse cuts than in those with one. It is concluded therefore that the loss of potassium is entirely through the cut ends or, if there is any loss through the rest of the surface this must be of a much smaller order of magnitude.

**A study of insulin hypersensitivity in hypophysectomized dogs.** I. H. SLATER (by invitation), R. C. DE BODO, H. F. WEISBERG (by invitation), S. P. KIANG (by invitation). *Department of Pharmacology, New York University College of Medicine.* Hypersensitivity to insulin is characteristic of hypophysectomized animals. The degree, development and possible modification of this abnormality was studied in a series of hypophysectomized dogs. The test dose of insulin (0.025 units/kgm intravenously) in normal dogs causes either no change in blood sugar or only a slight drop of short duration (recovery within 40 minutes). Two-three days after hypophysectomy a significantly increased response to insulin was apparent, the blood sugar consistently showed a drop and the recovery was delayed. Ten-fourteen days after operation the insulin hypoglycemia was more marked and the return to the basal level greatly delayed, often beyond four hours. During the fifth postoperative week and thereafter, insulin lowered the blood sugar to near convulsive levels with only rare recovery within four hours. Since the shape of the blood sugar curves obtained after insulin differ in normal and hypophysectomized dogs, the degree of hypersensitivity cannot be expressed numerically. Whereas in normal animals 1.5 units/kg insulin, 60 times the test dose, produces a hypoglycemia approximating the convulsive level, recovery occurs consistently within four hours. Furthermore, hypophysectomized animals have a significantly lower postabsorptive blood sugar, thus the insulin action starts at a lower level. The gradual development of the hypersensitivity suggests a relation to the secondary atrophy of the adrenal cortex and thyroid which might be preceded by a hypofunction undetectable histologically. Indeed, adrenal cortical extract modified somewhat the insulin induced hypoglycemic response.

**Some cutaneous responses to "reflex cooling"** DOUGLAS E. SMITH, WALTER C. RANDALL, and ALRICK B. HERTZMAN. *Department of Physiology, St. Louis University, School of Medicine.* Simultaneous estimations of skin blood flow and skin surface tem-

perature were made upon the finger, forearm, and cheek of normal men subjected to "reflex cooling." Immediately upon exposure of the legs and lower trunk to a cold water bath, the blood flow in the finger decreased markedly but underwent little change thereafter during the exposure. The skin temperature fell markedly but at a slower rate than the blood flow and continued to fall for the rest of the period of exposure to cold. The blood flow of the forearm decreased significantly immediately upon reflex cooling and either stayed low or rose to normal levels thereafter. At the same time the forearm skin temperature gradually fell. The responses of the cheek were variable, the flow being either increased or decreased. The cheek temperatures usually followed the direction of the cheek blood flow. During reflex cooling, oral temperature was observed to rise to a peak and then fall, reaching values below the control level. The relative degrees of the changes in blood flow and skin temperature in the various areas and their possible significance are discussed.

**Some toxic effects of acid and neutralized thiamine solutions.** JAY A. SMITH (by invitation), PIERO P. FOA, HARRIET R. WEINSTEIN (by invitation), A. SIDNEY LUDWIG (by invitation), and J. MARVIN WERTHEIM (by invitation). *Department of Physiology and Pharmacology, The Chicago Medical School, 710 S. Wolcott Avenue, Chicago, Illinois.* Thiamine hydrochloride solutions are acid. Upon injecting such solutions intravenously, respiration is depressed, and blood pressure decreases; these effects are transitory if artificial respiration is provided. It was desired to know if these changes were due to the thiamine or to the acidity of the solution. Thiamine hydrochloride solutions weakened or stopped the isolated perfused turtle heart; neutralized solutions depressed the heart slightly; acidified Ringer depressed the heart less than a thiamine solution at identical pH. Thiamine hydrochloride solutions caused vasodilation in the perfused rabbit ear in proportion to the concentration and the acidity. Neutralized thiamine solutions caused little change in the rate of perfusion. Thiamine hydrochloride depressed the heart in the heart-lung preparation only in concentrations sufficiently great to alter the pH of the blood appreciably. A thiamine hydrochloride solution (pH 3) intravenously to dogs altered the pH of the blood only slightly (from 7.54 to 7.52) within 20 seconds. The injection of identical quantities of a neutralized thiamine solution caused no shift in the pH. The effects on respiration and blood pressure were identical after injection of acid or neutralized thiamine solutions. It is concluded that in isolated systems, such as the turtle heart, rabbit ear, and heart-lung preparation, the effects of thiamine hydrochloride are due principally, although not exclusively, to the acidity of the solutions. In intact

dogs, on the other hand, the effects of thiamine are not due to the acidity of the solutions but to thiamine itself

**Experimental embolism of selected portions of pulmonary arterial bed** JOHN R. SMITH and MASUKI HARA (by invitation) *Department of Internal Medicine, Washington University School of Medicine, St. Louis* Using anesthetized, heparinized dogs with chests opened, fine catheters were introduced into the main pulmonary artery. The catheters could be guided into any lobar arterial branch. Minute or particulate emboli were then placed in a given lobar artery in selected quantity. Care was taken to prevent retrograde passage and scattering of emboli outside of the selected lung lobe. When small foreign bodies such as fine glass beads, poppy seeds or lycopodium spores were impacted in the lobar artery in large numbers, there were no significant effects upon cardiovascular dynamics. In contrast, the injection of very fine material, such as starch or barium sulfate suspensions, into the lobar artery produced a marked rise of pulmonary arterial pressure, a precipitous fall of systemic blood pressure, and intense distension of the right heart. Death occurred in 2 to 3 minutes. Since embolic particles such as beads and lycopodium produce no circulatory dynamic changes, and finer materials (starch or barium sulfate) produce severe circulatory changes, it is reasoned that pulmonary "capillary" emboli, affecting a restricted portion of the pulmonary vascular bed, may cause death with apparent pulmonary spasm and circulatory failure.

**The influence of thiourea and thiouracil on the response of rats to methyl chloride** WILLIE W. SMITH *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland* During an investigation of protective action by sulfur containing compounds, thiourea and thiouracil were tested. Month old rats in groups of 10 were given 20% casein diets 2 weeks preceding exposures to methyl chloride 2,000 p.p.m. 6 hours a day 6 days a week. Median survival time was about 10 days. With supplements of thiourea (0.46%) or thiouracil (0.77%), death occurred during or immediately after a single exposure. Again all rats died on one exposure with thiourea 0.05%, 8 died with thiouracil 0.1%, but only 2 with thiouracil 0.05%. With the supplements for only 3 days preceding exposure 9 on thiourea 0.06% and 8 on thiouracil 0.1% died. When thiourea (12 mg.) was injected intraperitoneally immediately before, 30 minutes after, or 2 hours after the exposure, all of the rats died. Of those injected 30 minutes after, 7 died within 2 hours evidencing extreme respiratory distress. Only 2 of the rats injected 18 hours after exposure died. With supplementary cystine, 0.45%, rats survive about 8 weeks. Thiourea 0.06% in this diet resulted in 100% mortality on a single exposure,

with thiouracil 0.1% 6 died within 2 days, 3 lived longer than 8 weeks. 6-Methyl 2-thiouracil, 6-propyl 2-thiouracil and sulfadiazine were fed for 3 days in molar concentration 5 times that of 0.1% thiouracil. Half of the rats on methylthiouracil died after 1 or 2 exposures, but the other compounds had little or no effect. The action of thiourea and thiouracil on methyl chloride exposed rats does not parallel their known effect on thyroid action.

**Excretion of bilirubin and bromsulfalein by the liver** W. J. SNAPE (by invitation), C. W. WIRTS (by invitation), L. L. MILLER and A. CANTAROW *Jefferson Medical College, Philadelphia, Pa.* Employing Thomas-type bile-fistula dogs, bile was collected continuously, in 15 minute samples, before, during and after intravenous injection of bilirubin and of bromsulfalein. The duration of injection and post injection periods was varied in different experiments. Serial determinations were made of the concentrations of bilirubin and bromsulfalein in the serum and of the volume, and total bile pigment and dye concentrations in the bile in 15 minute samples. After a single injection of 1 mg. bilirubin per kilogram, 48.9-89% of the amount injected was recovered in the bile in 3 hours. After a single injection of 5 mg. bromsulfalein per kilogram, 55.6-96.9% was recovered in the bile in 3 hours. After continuous injection (4-2 hours) of 0.7-5 mg. bilirubin per kilogram, 61-100% of the amount injected was recovered in the bile in 2-4 hours. After continuous injection of 4.5-12 mg. bromsulfalein per kilogram (2 hours), 48-74.7% was recovered in the bile in 4 hours. The data indicate that injected bilirubin and bromsulfalein are removed from the blood more rapidly than they are excreted in the bile. If the liver is the only organ involved in their removal from the blood, this implies a subsequent phase of temporary storage in that organ prior to passage into the bile.

**Facilitation produced by cerebellar stimulation** RAY S. SVIDER (by invitation) and H. W. MAGOUN *Department of Anatomy, Northwestern University, School of Medicine, Chicago, Illinois* In monkeys excitation of (a) anterior lobe lobulus simplex region and (b) paramedian lobule-tuber vermis pyramis region of the cerebellum produced facilitation of reflex activity and of movements evoked from the cerebral cortex. The most pronounced effects were noted when chloralose anesthesia was used and electrical stimuli of 200-300 cycles per second were applied to the cerebellum from 5-15 seconds before the elicitation of the movement. Facilitation occurred following destruction of the superior peduncle suggesting that the cerebellum is exerting its influence through facilitatory centers in the brain stem rather than through the activation of cerebral mechanisms. The distribution of cerebellar facilitatory areas in the monkey is similar to that of the tactile receiving areas and of the

areas yielding inhibition of movement in the cat. This similarity even extends to the somatotopic representation of body parts for, though overlapping was present, within zone (a) movements of the leg were facilitated from points rostral to those for the arm, while within zone (b) the reverse was the case.

**Oxygenation of the cord blood of breathing fetuses during a prolonged period.** FRANKLIN F. SNYDER, *Harvard Medical School*. The persistence of rhythmical respiratory movements in rabbit fetuses following their exposure in a saline bath with intact umbilical cord circulation, affords opportunity to sample the umbilical vein blood of breathing fetuses at various intervals after their escape from the uterus. The state of activity of the fetus and the degree of sensitivity to tactile stimuli can be correlated with the oxygen supply. *Following the inhibition of labor by the induction of ovulation and a fresh growth of lutein tissue near term*, the uterus is quiescent and the placenta remains attached even in fullterm and postmature rabbits. In fullterm fetuses under continuous observation for an hour or longer while showing rhythmical respiratory movements it is evident that the blood of the umbilical vein may remain bright red in contrast to the blue blood of the arteries. Determination of the oxygen content in 28 blood samples obtained from the umbilical veins of 13 fullterm fetuses showed that at the beginning and at the end of one hour, the oxygen content averaged 11 volumes per cent, the percentage saturation with oxygen averaged 73 at the beginning and 78 at the end of one hour. The carbon dioxide content of the blood was lower at the end of an hour of exposure in the saline bath than at the beginning.

**Pulmonary function as affected by operative positions.** A. SOKALCHUK (by invitation), D. ELIIS (by invitation) and F. M. GREISHEIMER, *Department of Physiology, Temple University School of Medicine*. We have determined rate, tidal volume, oxygen use, maximal breathing capacity and vital capacity, and calculated from these minute volume of respiration, ventilatory equivalent and per cent reserve. The twelve positions studied are those used in the operating room of Temple University Hospital. Each subject has been observed three times in each position. The supine position is used as the control, and the results in the other positions are compared with this. The following trends have been noted in the subjects studied to date. The rate is faster in the sitting and jack knife positions. The tidal volume is lower in the sitting, both lateral and both kidney positions. It is higher in the Trendelenburg position. The oxygen use is greater in the sitting, prone and lithotomy positions. The maximal breathing capacity is greater in the sitting position. It is decreased in the Trendelenburg, both lateral, both kidney and gall

bladder positions. The vital capacity is greater in the sitting position. It is lower in the prone, Trendelenburg, right lateral, both kidney, gall bladder, lithotomy and jack-knife positions. The minute volume of respiration is decreased in the reverse Trendelenburg, both kidney and gall bladder positions. The ventilatory equivalent is lower in the sitting, Trendelenburg, left lateral, both kidney and lithotomy positions. The per cent reserve is lower only in the lithotomy position.

**The mechanism of nitrous oxide analgesia.** R. R. SOVENSCHEIN, R. JAMISON, L. LOVSETH, W. CASSELLS (by invitation) and A. C. IVY, *Depts of Clinical Science and Anesthesia, University of Illinois College of Medicine, Chicago*. The pain threshold-raising action of nitrous oxide, in concentrations of 10% to 40% in oxygen, was studied by the method of electrical stimulation of the tooth pulp (Goetzl, F. R., Burrill, D. L., and Ivy, A. C. *Quart Bull, Northwestern U. Med Sch*, 17: 280, 1943). Control and test determinations were made at one to two minute intervals. Psychomotor activity was tested before and during gas administration, by means of the Johnson Code Test.

Forty one tests on eighteen subjects revealed a significant rise in threshold of 0.30 to 0.45 volts (approximately 25%), at concentrations of 20%, 33%, and 40%  $N_2O$ , results at 10% were variable. Results of twenty-two psychomotor tests during gas administration showed an increase of 14% to 10% in the standard test time at concentrations of 10%, 20%, 33%, and 40%. Readings on four occasions with the Millikan oxymeter, at 33%  $N_2O$ , showed no change in oxygen saturation, over the control readings.

It is concluded that the analgesic action of nitrous oxide, as a typical general anesthetic agent, is associated with a diminution in psychomotor activity. It appears likely that the analgesic action may be secondary to the latter factor.

**Performance in warm environments following hemorrhage, albumin infusion, bed rest and exposure to cold.** C. R. SPEALMAN, E. W. BIRBY (by invitation), J. LARUE WILEY (by invitation), MICHAEL NEWTON (by invitation) and H. C. BAZETT, *Department of Physiology, University of Pennsylvania, Philadelphia*. Deterioration of ability in certain physical activities (endurance and tilt table tests) can be detected at normal temperatures following the removal by venesection of 500 to 1000 cc of blood (Karpovitch and Millman, *Res Quart* 13: 166, 1942; Green and Metheny, *Surg Gyn Obs* 84: 1045, 1947). One would expect even more pronounced effects with unacclimatized individuals in very warm environments which necessitate the physiologic stress of maintaining blood volume adequate for a vascular system enlarged by dilatation of blood vessels in the skin. In our experiments, performance (tilt table, bi



cycle ergometer, Crampton test) in the heat ( $33^{\circ}\text{C}$ , D B,  $28-29^{\circ}\text{C}$ , W B, wind velocity 50 cm/sec) became markedly poorer shortly after the withdrawal of 500 cc of blood and remained so for several days. Removal of 200 cc of blood also resulted in decreased ability to perform. Evidence that the causal factor was a decrease in blood volume rather than a decrease in hemoglobin was obtained in experiments in which performance was similarly affected by decreasing plasma volume alone (induced by bed rest and exposure to cold). Furthermore, increasing blood volume by infusing serum albumin (in an amount equivalent to 500 cc of plasma) improved performance in the heat. Eight normal healthy young men served as subjects for these experiments.

**Electroencephalographic studies following thalamic lesions in humans.** E. A. SPIEGEL and H. T. WICKS (by invitation), *Dept of Experimental Neurology, Temple Univ School of Medicine, Phila, Pa.* Circumscribed lesions were placed by electrocoagulation in the region of the medial nuclei of the thalamus (medial thalamotomy) for treatment of some mental disorders associated with emotional tension, or thalamotomy was combined with lesion of the spinothalamic system in the mid brain in cases of intractable pain. The stereotaxic technique previously described by us (Science 106:349, 1947) was used for introducing the coagulating electrode into the brain. The electroencephalogram was recorded from the scalp before and after operation. In some instances also a basal electrode was applied through the nose so that it touched the posterior wall of the nasopharynx. A comparison of the electrograms with those obtained after prefrontal lobotomy showed that the disturbance of cortical function as far as revealed by the electrical discharges is much smaller after thalamotomy than after prefrontal lobotomy. However in some instances, at least a transitory slowing of the brain waves, particularly in the frontal leads, could be demonstrated, apparently due to loss or diminution of the impulses from the medial nuclei to the frontal poles. The results are compared with similar studies on monkeys.

**Protection by substrate against inhibition of enzymatic adaptation by protein denaturants.** S. SPIEGELMAN and JOHN M. REINER, *Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis, Mo.* Previous studies (Arch Biochem, 13:113, 1947) have indicated that enzymatic adaptation involves a modification in the protein components of the cell. One of the problems raised by this phenomenon is the nature of the precursor which is converted into active enzyme by the presence of substrate. In particular, the question arises as to how close a relation exists between the precursor and the final active enzyme. A method of throwing some light

on this problem was provided by the finding that protein denaturants, such as urea, guanidine and desoxycholate exerted strong inhibitory effects on the adaptive process. These experiments indicated that a denaturable molecule was an important component of the adaptive system. If this component was sufficiently similar to the active enzyme so that it could bind with substrate, it would be expected that the presence of substrate would protect it from inactivation. Experiments performed to test this possibility confirmed the expectation. Unadapted cells treated with any of these agents previous to incubation with substrate took two-three times as long to show evidences of enzyme formation. If, however, the substrate was added first and then the denaturing agent, no inhibition of enzyme formation was observed. These experiments would therefore indicate that a component, present in unadapted cells and formed during enzymatic adaptation, although not enzymatically active, can combine with substrate. The combination with substrate protects the component against inactivation by protein denaturants.

**Studies on diffusion respiration v The hemoglobin-oxygen pump.** JOSEPH N. SPENCER (by invitation), WILLIAM B. DRAPER, THOMAS M. PARRY (by invitation) and RICHARD W. WHITEHEAD, *From the Department of Physiology and Pharmacology, University of Colorado Medical Center.* Draper and Whitehead (1944) reported that animals placed in respiratory arrest in an atmosphere of oxygen may survive for considerable periods. They attributed their results to the continuous *vis a fronte* exerted on the atmosphere of the respiratory spaces by the hemoglobin oxygen pump. The functional importance of this pump is illustrated by the following experiments. Dogs, following denitrogenation, were connected with an oxygen filled, sensitive spirometer, placed in respiratory arrest with thiopental or curare and the oxygen uptake determined. In five experiments lasting forty five minutes or longer, there was essentially no difference in oxygen uptake between the animals receiving thiopental and those receiving curare. With respiratory arrest, oxygen uptake decreased approximately 11 per cent. This level was maintained for thirty minutes, then gradually fell to reach a level 30% less than the control during the last few minutes of life, ceasing abruptly with cardiac arrest. In a second series of five experiments the spirometer bell was seized following respiratory arrest. The maximum negative intrapulmonary pressure developed in the series exceeded 10 cm of water. In all instances where the spirometer bell was seized, death was associated with a profound collapse of the lungs. In a third series of experiments the animals, after respiratory arrest, were connected to a spirometer filled with

a carbon monoxide oxygen mixture. The atmosphere was moved en masse and carbon monoxide appeared in the blood in proportion to its concentration in the spirometer.

**A mass spectrometer for the rapid, continuous analysis of respiratory gases.** By RALPH W. STACY, (by invitation), JACK A. HUNTER, (by invitation), and F. A. HITCHCOCK. *The Laboratory of Aviation Physiology, The Ohio State University, Columbus.* To make possible a satisfactory study of the changes in composition of alveolar air that occur following rapidly induced changes in ambient pressure it was necessary to develop an analytical instrument that would record accurately rapid changes in composition of the air leaving the lungs. We have, therefore, designed and constructed a mass spectrometer of the Neir type, specially adapted for the measurement of respiratory gases. The instrument makes possible the simultaneous analysis for oxygen, nitrogen, and carbon dioxide. A special calibrating arrangement permits the quantitation of each of these components. The instrument records the concentrations of all three components on a single photographic film. The ion source is so constructed that analysis is continuous. The time required for recording full scale concentration change is less than two tenths of a second. The analytical error of the instrument is less than two per cent of full scale in normal use. An expanded scale provision has been incorporated which magnifies the changes, thus increasing the accuracy. Preliminary analyses of alveolar air expulsions have not verified the existence of an oxygen trough. This problem is being studied further.

**Effect of anoxia on the glucose tolerance curve of dogs.** J. CLIFFORD STICKNEY, DAVID W. NORTHUP and EDWARD J. VAN LIERE. *Dept. Physiology, School of Medicine, West Virginia Univ., Morgantown.* Seven fasted, unanesthetized dogs were given intravenously 1.5 g glucose per kilogram body weight. Blood sugar concentrations were determined by the Folin Wu method on samples drawn immediately before and at 15, 30, 60 and 90 minutes after the injection. Glucose tolerance curves were likewise determined while the dogs were subjected (except for brief sampling periods) to a barometric pressure of 254 mm Hg. The hyperglycemic response of the dogs to the same barometric pressure was also determined on samples drawn at the above time intervals. In most cases the response of each dog in the three types of experiment was determined three times, but at weekly intervals to diminish the cumulative effects of repeated glucose injections as well as of repeated exposures to anoxia. Fifteen minutes following glucose injection, the blood sugar values averaged 266 per cent above the control. At 60 min they had fallen uniformly to 29 per cent above, and at 90 min were at

or below the control. Anoxia tended to reduce the height of the 15-minute maximum of the glucose tolerance curve, but slowed the rate of fall beyond that point so that the 90 min value was still 33 per cent above the control. Anoxia alone, caused a 32 per cent rise in blood sugar which fell off gradually to 19 per cent at 90 min.

**Direct experimental comparison of several surface temperature measuring devices.** ALICE M. SROLL (by invitation) and JAMES D. HARDY. *From the Russell Sage Institute of Pathology, The New York Hospital, and the Department of Physiology, Cornell University Medical College, New York, N. Y.* This paper presents comparative data on several surface temperature measuring devices used in a variety of experimental conditions simulating natural climatic environments. The instruments studied included the radiometer, a resistance wire thermometer, surface pyrometers, several different types of wire thermocouples and mercury in glass thermometers. An apparatus for providing a standard surface whose temperature could be determined within  $\pm 0.05^\circ\text{C}$  under all experimental conditions is described. The simulated environments employed were normal room conditions, forced convection, infrared radiation, "sunlight" radiation, and "sunlight" plus forced convection. The results of this study indicate that the radiometer is the most dependable of the instruments studied. With this instrument an accuracy of  $\pm 0.1^\circ\text{C}$  was maintained under all conditions whereas measurements made with other instruments of equal accuracy under room conditions were affected seriously by radiation and forced convection. The advantages and disadvantages in the application of each instrument to the accurate measure of skin temperature are discussed on the basis of the experimental findings.

**Olfactory acuity and appetite effects of bitter tonics on olfactory acuity in normal human subjects.** FREYA STONL (by invitation) and FRANZ R. GOETZL. *Department of Medical Research, Permanente Foundation, Oakland, California.* Olfactory thresholds (Elsberg's method) were determined in normal individuals daily, at regular intervals. On test days the subjects' statements regarding appetite and their caloric intake were recorded. Meals were found to be preceded by a period of increasing and followed by one of increasing olfactory acuity. The precibal increase in olfactory acuity could be prevented by intercalary ingestion of food, the decrease in that acuity by omission of meals. It is suggested that by estimating diurnal variations in olfactory acuity measures may be found for the sensations of appetite and satiety. Benzedrine was observed to produce decrease in olfactory acuity, decrease in the intensity of the sensation of appetite, decrease in caloric

value of freely selected meals and, also, a sensation of satiety. Bitter tonics ingested between meals failed to influence precibal increase in olfactory acuity, intensity of the sensation of appetite, and caloric values of succeeding meals. The absence of effects of bitter tonics may be explained by assuming refractoriness to augmentation in normal individuals of precibal increase in olfactory acuity and intensity of the sensation of appetite, both presumably of optimal magnitude. When ingested during meals, however, bitter tonics were found to prevent the postcibal decrease in olfactory acuity and to render incapable freely selected meals of bringing about the conversion of the sensation of appetite into one of satiety.

The question whether the effects of bitter tonics described are related to appetite stimulating effectiveness commonly ascribed to bitter agents is being subjected to further investigation.

**Adjustment of caloric intake of rats to dietary change.** JACK L. STROMINGER (by invitation) and JOHN R. BROBECK *Lab of Physiology, Yale Univ School of Medicine, New Haven, Conn.* When 43% fat was added to the calf meal diet of 222 gm. male albino rats, during the first five days they ate 132% of the calories taken by controls. During the next 19 days they ate the same number of calories as controls. When 43% carbohydrate was added to the calf meal diet of similar rats, during the first five days they ate 91% of the calories of controls. During the next 19 days they ate the same number of calories as controls. When 30% or 60% fat was added to the calf meal diet of 101 gm. male albino rats, during the first 5 days they ate 117% of the calories of controls. For the next 17 days they ate 148% of the calories of controls. When 30% or 60% carbohydrate was added to the calf meal diet of similar animals, during the 22 day period they ate the same number of calories as controls. When 50% fat was added to the calf meal diet of obese female rats with hypothalamic hyperphagia, for 21 days they ate about 200% of the calories they had taken on the calf meal diet. Female unoperated controls ate about 200% for the first day, and thereafter diminished their caloric intake until by 5-7 days they were eating the same number of calories they had taken on the calf meal diet. The operated animals showed marked acceleration of weight gain during this period, while the controls gained at a normal or slightly diminished rate.

**The recovery period after resuscitation.** H. G. SWANN and MARSHALL BRUCER (by invitation) *Dept. of Physiology, University of Texas Medical School, Galveston.* Dogs were subjected to the anoxia of breathing pure nitrogen or 2-43% oxygen in nitrogen or 1% carbon monoxide in air. When death was imminent, various measures to ventilate the lungs were instituted (see M. Brucer and H. G. Swann, this journal) in order to resuscitate the

animals. The first sign of recovery was a sharp rise in systolic blood pressure, but not necessarily a rise in diastolic pressure. Soon thereafter breathing was reinstated. The first rise in blood pressure occurred within 100 seconds or not at all, even though the pulmonary ventilation was continued for 20 minutes. If good heart action was not resumed within 100 seconds, then the attempted resuscitation failed and further pulmonary ventilation was futile. When cardiac massage as well as artificial respiration was instituted, recovery from lower levels was accomplished. However the return of blood pressure and respiration was slower. In all but one instance, the animals showed various degrees of dementia, — i. e. they were blind or comatose or exhibited signs of decerebrate rigidity. This dementia, presumably due to the anoxic experience, was also frequently observed in carbon monoxide-poisoned dogs which were resuscitated only by measures ventilating the lungs.

**Principles of protection against the effects of negative "G."** (Motion Picture) HENRY M. SWEENEY and Biophysics Branch personnel *Aero Medical Lab., Air Materiel Command, Wright Field, Dayton, Ohio.* A colored motion picture was made to show methods of protecting against the effects of negative acceleration. The development of hemorrhage and petechiae in the sinuses and conjunctivae is shown in man and animals. Motion pictures of the blood vessels of the brain of a monkey with a lucite calvarium under g show that within the closed box of the skull fluid counter-pressure protects against blood vessel rupture. The technique of radial arterial and frontal vein cannulation using an Ungar type double needle is demonstrated together with the use of a Gauer-Wetterer inductance pressure gauge for measurement of venous and arterial pressure in man while undergoing negative acceleration. This film shows pictures of humans undergoing abrupt accelerations demonstrating the greater tolerance achieved by shortening the period of application of g. The principle of protection afforded by inclination of the long axis of the body to the direction of the g-force is demonstrated.

**The effect of adrenal cortical extract on adrenal response to total body X-irradiation.** MARGUERITE N. SWIFT, HARVEY M. PATT and ELLA B. TIERRE (introduced by AUSTIN M. BRUES) *Biology Division, Argonne National Laboratory, Chicago, Illinois.* The transient 20-50% decrease in adrenal total cholesterol observed in albino male rats 3 hours after a midlethal total body exposure to X radiation (Patt et al. *Am. J. Physiol.* 150:480, 1947) is largely prevented by subcutaneous injection of adrenal cortical extract (Wilson). Total doses of 0.3-1.8 cc./200 gm. rat were similar in effect, with no graded response over this range. This is additional evidence that the early chole-

sterol fall reflects increased adrenal activity. In order to investigate the nature of the marked rise (70-100%) in adrenal cholesterol observed 3-7 days after a midlethal exposure to X-radiation, a second similar exposure was administered to rats 1 day after the first. No fall in adrenal cholesterol occurred by 3 hours after the second exposure, and the cholesterol level at this time was unaffected by cortical extract dosage sufficient to prevent the early decrease seen after a single X-ray exposure. Elevated adrenal cholesterol is reported to occur only after prolonged stimulation by adrenotrophic hormone, and may in this case also represent overstimulation in excess of cortical hormone demand. Rats given 0.1 cc. of extract three times daily, an amount sufficient to prevent the early cholesterol fall, show adrenal cholesterol changes of slightly lesser degree than do controls over a 15 day post-irradiation period. Survival is not enhanced by this treatment. Larger amounts of extract might possibly prove effective since increased survival has been reported after X irradiation by shielding the adrenals (Craver, Fed. Proc. 6:319, 1947).

**Silicone implantations in the rat.** VIRNÉ W. SWIGERT (by invitation), HAMILTON R. FISHBACK (by invitation), LILLIAN E. CISLER (by invitation), and FREDERIC T. JUNG, *Northwestern University Medical School, Chicago 11, Ill.* Weighed samples of two types of silicone were implanted aseptically into the abdominal cavities of white rats. The first, a solid type of silicone, similar in appearance to white rubber, was in the form of cylindrical pieces, about 6 mm. in diameter. The second type was a variety called "bouncing putty" because it flows like an opaque grayish liquid with low surface tension, but slowly, and bounces if dropped on the floor. The bouncing putty was used in a series of 61 operations beginning 1946 Jan. 28; the solid type was used in only 4 rats beginning 1946 Feb. 18. The animals were sacrificed for necropsy at times varying from 2 months to a year. There were no indications in the behavior of the animals, or in their weight curves, or in the necropsy findings, that absorption or any chemical change in the silicones had occurred during that time. The bouncing putty remained recognizable as grayish deposits of varying size, sometimes very finely divided and often widespread, on both parietal and visceral aspects of the peritoneum. The silicone was not transported to deeper or remoter structures, but remained on the surface. It was enveloped in a thin layer of connective tissue that contained occasional giant cells and showed signs of mild chronic reaction. One rat developed a tumor that grew rapidly until it constituted 20% of the body weight, since the tumor was extra-abdominal, its significance was debatable.

**The chemical composition of regenerating rat liver: the influence of ovariectomy.** CLARA M.

SZILGO and SIDNEY ROBERTS, *Department of Physiological Chemistry, Yale University, New Haven.* In the adult female rat, the liver stump remaining after removal of two thirds of the liver doubled its weight within two days. During the first day, the percentage of fat in the liver reached a maximum of 12% (normal, 6%) and the water content dropped to 67% (normal, 71%). The change in percent protein was apparently relatively small. On a body weight basis, total liver lipid returned to normal within 2 days (250 to 300 mgm. per 100 gm.). Subsequently, as liver restoration progressed, the percentage of water and fat approached normal levels. Rats ovariectomized two weeks prior to partial hepatectomy exhibited overall changes similar to those observed in non ovariectomized animals. A profound difference was, however, noted during the first 8 to 10 hours following operation. In control animals, hepatic fat mobilization occurred essentially at a constant rate for the entire first day (ca. 8 mgm. per 100 gm. body wt. per hr.), a concomitant decrease in liver water was observed. On the other hand, in the ovariectomized group there was an initial lag (first 10 hours) in fat deposition, accompanied by a rapid uptake of water (70.6% to 72.5%). Thereafter, the rate of fat mobilization was similar in both groups, so that the maximum lipid concentration reached in the ovariectomized group was only about 10%. Preliminary observations indicated that the administration of estrogen to the ovariectomized rat immediately after partial hepatectomy may restore the initial rate of fat transport to the liver to normal.

**Effects of the cold pressor test on glomerular filtration and effective renal plasma flow.** P. J. TALSO (by invitation), A. P. CROSLLEY, JR. (by invitation), and R. W. CLARKE, *Medical Department Field Research Laboratory, Fort Knox, Ky.* Studies were made of the effects of the cold pressor test on renal function. Seven male volunteers who had no history of renal disease served as subjects. Glomerular filtration (as measured by mannitol clearance) and effective renal plasma flow (as measured by sodium para-aminohippurate clearance) were determined before, during and after immersion of the foot in ice water at 1°C. for 15 minutes. In 6 of 7 subjects both glomerular filtration rate and effective renal plasma flow decreased either during the application of the cold stimulus or within approximately 30 minutes thereafter. In no subject did the effect persist longer. The average decreases in glomerular filtration rate and effective renal plasma flow, as compared with the control values, were 14% and 21%, respectively.

**The behavior of certain characteristics related to exhausting work during recovery from semi-starvation.** HENRY LONGSTREET TAYLOR, AUSTIN HENSCHEL, JOSEF BROZKA, and ANCEL KEYS, *Laboratory of Physiological Hygiene, University*

of Minnesota, Minneapolis Six men who lost 24 per cent of their body weight during a six months regimen on a famine diet characteristic of Western Europe were studied during the 20 weeks of recovery necessary to regain the original body weight. Maximal oxygen intake, 12 minute recovery lactate and 10-minute oxygen debt were studied during and after a 3 minute run at 7 miles per hour and at a grade which depended on the capacity of the subject. The Harvard Fitness Test was used and measurements of strength were made with the hand and back dynamometer. Loss of function from 20 to 70 per cent of the control value was noted in these measurements after semi-starvation. Recovery was slow. After 12 weeks, the Harvard Fitness Test score had recovered 58 per cent and the maximal oxygen intake 42 per cent of the semi starvation loss. After 20 weeks of re-feeding, when the men had regained their weight, the maximal oxygen debt had regained their normal values. The time and score of the Harvard Fitness Test recovered only 78.0 and 72.0 per cent of their respective losses, while the hand and back dynamometers had regained only 46 and 86 per cent of the strength lost. It is suggested that the poor return of strength was in a large part responsible for the failure of endurance, as measured by the Harvard Fitness Test, to recover as rapidly as the body weight.

**Effects of denervation on experimental renal hypertension** ROBERT D. TAYLOR, A. C. CORCORAN and IRVING H. PAGE. *From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio*. High caudal or spinal anesthesia at D5 in patients suffering from essential hypertension commonly results in a decrease of arterial pressure to normal levels and an increase of minimal renal plasma flow to average 1.2 times normal. It follows that one effect of such anesthesia is a considerable decrease in renal vascular resistance. In attempting to define this phenomenon further observations were made in dogs with experimental renal hypertension due to perinephritis. Of 11 dogs given spinal or epidural anesthesia to levels causing paralysis of the nictitating membrane, arterial pressure was decreased in all (means from 172 control to 122 mm Hg during anesthesia) and minimal renal plasma flow increased on the average by 10 per cent in 9. Renal plasma flow was decreased in 2 dogs in which control levels were maintained for 36 hours. Thus, in dogs, as in patients, spinal or epidural anesthesia usually induces renal vasodilation. That this vasodilation was not due to the systemic effects of the anesthetics used (procaine and metycaine) was shown in 4 experiments in which these drugs were injected into lumbar muscles and 2 in which they

were given intravenously. The effect is therefore due to the denervation caused by local drug action. Arterial pressure and renal plasma flow were determined in 2 dogs after spinal section by ligature and caudal pithing. Section at D5 did not alter these functions but section at C6 or C8 had the qualitative effects of high spinal or epidural anesthesia. Similarly, autonomic denervation by intravenous infusion of 2 hypertensive dogs with ETAN decreased arterial pressure while renal plasma flow was unchanged or slightly increased.

It is concluded that maintenance of experimental renal, as of clinical arterial hypertension, is partially dependent on an unidentified vasomotor outflow from the upper thoracic spinal cord.

**Response of deafferented neurones of spinal cord to impulses reaching them from higher levels of central nervous system** R. D. TEASDALE (by invitation) and G. W. STAVRAKIS. *Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada*. Decentralization is known to increase the sensitivity of spinal neurones to chemical stimulating agents (Cannon, *Am J Med Sci* 198, 737, 1939; Stavrakis, *Am J Physiol* 150, 37, 1947). It was shown recently that deafferentation leads to similar results (Stavrakis and Drake, *Federat Proc* 6, 211, 1947). The latter finding provided a method by which to determine whether the increased irritability of partially deafferented neurones is limited to a greater effectiveness of chemical agents upon such neurones, or whether they will respond more readily to nerve impulses reaching them along pyramidal tracts and other descending systems of nerve fibres as well.

In cats, 10 days to 3 months after aseptic deafferentation of the hind limb, decerebration and electric stimulation of the region of the cerebral peduncles on the transected surface of the brain was carried out. The threshold of the response to faradic stimulation of the corresponding cerebral peduncle was markedly lowered by preceding deafferentation of the limb. Furthermore, the movements observed in the deafferented limb on stimulation of the cerebral peduncle were sustained after the end of stimulation over longer periods of time than movements of the intact limb. A study of the labyrinthine and neck reflexes in deafferented fore and hind limbs of chronic animals is in progress and a demonstrable hyperactivity of reflex mechanisms is found to be present. All these results indicate that partial denervation increases the irritability of the neurones in the central nervous system, not only to chemical stimulating agents, but to physiological nerve impulses traversing intact synapses.

**Critical flicker frequency in defective fields of vision** HANS-LUKAS TEUBER (by invitation) and MORRIS B. BENDER. *From the Psychophysiological Laboratory, Department of Neurology, New York*

*University, College of Medicine* In 28 Naval casualties with penetrating wounds of the occiput, critical frequencies for fusion of visual flicker (cff) were significantly below those of 20 normal controls. This was found in the center of the visual fields and at increasingly eccentric points in each quadrant. In the defective fields, the per cent loss in cff (as compared with corresponding normal values) was minimal in a zone  $10^\circ$  from the fixation point. With increasing eccentricity the loss in cff increased. Maximal reduction of cff occurred in homonymous half fields or quadrants. In the same areas of greatest depression of cff there were maximal disturbances in perception of apparent (stroboscopic) and actual movement, the apparent speed of objects moving through these areas of maximum depression of cff was increased. This was demonstrated by moving targets at variable rates through different areas of the field, and by having the subjects manipulate the target speeds until the speeds appeared subjectively equal. Thus objects moving at various standard speeds appeared to the subjects with trauma, but not to the controls, as either abnormally fast, elongated or multiplied. It was concluded: 1) neural rather than photochemical factors limit flicker perception in these brain injured subjects, 2) the limiting factor is an (abnormally slow) intermittence of cerebral function subserving vision, 3) one and the same functional mechanism determines perception of flicker, apparent motion and actual motion.

**Comparison of mannitol and thiocyanate volumes in several patho-physiological conditions**  
C P THARP (introduced by R R OVERMAN) *Department of Physiology, University of Tennessee, College of Medicine, Memphis, Tennessee* Recent reports have indicated that SCN fails to remain primarily an extracellular anion in malaria and certain other diseases (J Lab Clin Med 31:1170, 1946). We have felt obliged, therefore, to compare the volume of distribution of mannitol, a substance of molecular size, with that of SCN in several patho-physiological conditions. Simultaneous measurements of these volumes were consequently made with the following average findings expressed as cc/kgm body weight: (1) Normal pregnant human patients, mannitol 297 cc, SCN 282 cc. (2) Pregnant patients with eclampsia, mannitol 296 cc, SCN 281 cc. (3) Normal dogs, mannitol 287 cc, SCN 293 cc. (4) Dogs severely dehydrated by water and food deprivation for approximately 20 days, mannitol 366 cc, SCN 356 cc. It is evident from these data that mannitol and thiocyanate are diluted by approximately the same fluid volume in the normal condition, and that they continue to occupy approximately the same space in certain severe patho-physiological alterations.

In (1) the post-pyrexia state after several daily

artificial temperature elevations and (2) following unilateral adrenalectomy, however, mannitol and SCN are not diluted by the same proportion of body water of the dog. In these conditions the SCN space may become as much as 61 per cent greater than the mannitol space, which remains relatively constant. It may be inferred, therefore, that while mannitol continues to be diluted only by extracellular water, SCN may enter tissue cells and be diluted by an increasing proportion of intracellular water in these conditions.

**Injury potential in potassium depleted frog muscle** JULIAN M TOBIAS *University of Chicago Department of Physiology and Toxicity Laboratory* In an attempt to further evaluate the assumed role of potassium in the genesis of certain bio potentials, the injury potential has been studied, mainly by the crush technique in frog sartorii after soaking in potassium free solutions to remove potassium. Muscles soaked in normal Ringer tend to show a higher injury potential than those soaked in potassium free Ringer. In muscles soaked in normal Ringer there is a tendency, though slight, for the E M F to parallel the potassium content. In muscles soaked in potassium free Ringer there is no correlation between E M F and total potassium. Muscles soaked in distilled water for 5-48 hours become potassium free and lose most of their sodium. Interestingly, sodium seems the more tenaciously held. Such muscles, though potassium free, may exhibit potentials up to 30 mv though most are lower, 9-15 mv. This potential rapidly falls to zero when outside potassium is increased to 0.1 N on the uncrushed surface of the muscle. The usual explanation that such a potential fall is due to levelling an intracellular-extracellular potassium gradient cannot apply since there is no potassium in the muscle to begin with. A few experiments with the micro capillary electrode of Gerard et al (with the generous help of Mr Gilbert Ling) show the potential of the distilled water soaked muscle, truly measured between the cell interior and exterior, may be reversed from the usual orientation to as high as 40 mv although the same muscle shows a 10-15 mv E M F of the usual orientation by the crush technique.

**Sodium and potassium in insects, larvae, pupae and adults** JULIAN M TOBIAS *University of Chicago Department of Physiology and Toxicity Laboratory* The high haemolymph potassium and low sodium which have been reported for certain insects have important implications for the functioning of irritable tissues, genesis of membrane potentials and the mechanisms of potassium accumulation and relative sodium exclusion by cells. In roach (*Periplaneta americana*) and grasshopper (*Rumex micropus*) haemolymph respectively, sodium (107 and 64 mM) exceeds potassium (17.3 and 18 mM), but the Na/K ratio is small (6.2 and

3.6) compared to that in the frog (41.6) and man (29.2). This is primarily because of the high potassium in the insects. In the silkworm (*Bombyx mori*) larva with a still higher potassium (40 mM) and very low sodium (14 mM) the ratio is less than unity (0.35). Larval haemolymph sodium (14 mM) in the silkworm may be in simple diffusion equilibrium with the sodium of the food eaten (mulberry leaf) (18 mM). Most interestingly, the entire silkworm pupa seems to contain no sodium at all or vanishingly small quantities of it. This is not true of *Cecropia* or *Cynthia* pupae. Data for the roach, grasshopper and silkworm suggest that the low sodium and high potassium are coincidental with the vegetarian food habit but not immediately causally related to it. Roach nerve and muscle can apparently function normally in a body fluid whose potassium concentration is as high as 49.2 mM. The evidence shows that tolerance for such high potassium levels need not be on a basis of potassium binding as has been postulated in the past.

**Lipid inhibitors and accelerators of blood coagulation in extracts of human brain.** L. M. TOCANTINS, R. T. CARROLL (by invitation) and THOMAS MCBRIDE (by invitation). *Division of Hematology, Dept. of Medicine, Jefferson Medical College, Philadelphia, Pa.* Concentrated aqueous extracts of human brain and aqueous suspensions of ether extracted lipids of the same organ contain mixtures of inhibitors and accelerators of blood coagulation. A potent lipid inhibitor, soluble in acetone, ether, alcohol, chloroform and carbon tetrachloride, heat labile (destroyed after 10 minutes at 70°C) may be separated from the clot accelerating cephalin fraction, when freed of it, the potency of the inhibitor becomes greatly magnified. The inhibitor exerts its action on the first phase of coagulation (availability and utilization of thromboplastin), it delays the activation of prothrombin by strong thromboplastin and cephalin preparations, and enhances the antithromboplastic (and anticephalin) activities of normal plasma. The inhibitor is not heparin, is not inactivated by protamine, has no antithrombin or antifibrinogen action and differs in its behavior to heat, its solubility and method of extraction from a lipid inhibitor previously isolated (Chargaff 1937) from the spleen and other organs.

**Some effects of anticholinesterases upon frog sciatic nerve.** JAMES E. P. TOMAN. *Dept. of Physiology, University of Utah School of Medicine, Salt Lake City, Utah.* Confirming the observations of Lorente de No (*Studies Rockefeller Inst. Med. Research* 131: 195-243, 1947), it was found that diisopropyl fluorophosphonate (DFP) or eserine sulfate could prevent the conduction block produced by prolonged exposure to isotonic solutions of acetylcholine chloride. Eserine 0.001 M was more

effective than the same concentration of DFP, but DFP protection persisted after repeated washing in Ringer's solution and was correlated with persistent reduction in cholinesterase activity. Eserine prevented the irreversible effects of DFP exposure. The results illustrate typical anticholinesterasic actions of DFP and eserine in concentrations which were without demonstrable direct effect upon nerve, and further substantiate the lack of effect of unhydrolyzed acetylcholine upon frog nerve. In contrast to the anticholinesterasic actions of DFP and eserine, direct effects (increased threshold, decreased conduction velocity) at higher concentrations (0.025 M) were more pronounced but more quickly reversible with DFP than with eserine. The relation between conduction velocity  $v$  in m/sec and threshold  $\tau$  in arbitrary units was found to be  $v = L(2I - \tau)/IT$ , where the constant  $I$  is threshold at moment of conduction failure, and  $L$  and  $T$  are the space and time constants of the fibers.

**Effect of acetylcholine, caffeine, and alkaloids on activity of muscle adenosinetriphosphatase.** CLARA TORDA and HAROLD G. WOLFF. *New York Hospital and Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York, N. Y.* The effect of acetylcholine and other chemical agents known to modify the shortening of muscle (alkaloids, caffeine) on the activity of muscle adenosinetriphosphatase was investigated. Actin-free myosin and actomyosin served as the source of the enzyme and adenosinetriphosphate (ATP) as the substrate (method of Szent Györgyi and the modified method of Greenstein and Edsall as described by Singher and Meister). Acetylcholine, in low concentrations, increased the activity of the enzyme in splitting one phosphate from ATP. Caffeine, d-tubocurarine, and potassium, in concentrations corresponding to those inducing muscle contracture, decreased the activity of the enzyme. The other substances used (atropine, ephedrine, morphine, physostigmine, prostigmine, quinine, strychnine, and veratrine (in concentrations not interfering with the phosphorus determination)) did not modify the activity of muscle adenosinetriphosphatase. These results indicate that acetylcholine, by augmenting the activity of adenosinetriphosphatase, may contribute to a more adequate recovery of the muscle.

**Experimental A-V block, method and discussion.** WILLIAM G. TURMAN (by invitation) and JANE SANDS ROBB. *Department of Pharmacology, Syracuse University, College of Medicine.* Desiring to record a  $v$  bundle depolarization, sharp pointed shielded electrodes of silver chloride coated silver wire were fused into small glass tubes. These could be plunged into anterior or posterior septal regions or could be forced through the right ventricular wall and brought to rest near or upon the bundle

of His. In this additional position pressure usually produced temporary block. From direct surface or from indirect leads, potentials not reflected from the septal leads may appear and vice versa. In general, basal septal electrocardiograms have a more complex wave form than those from other heart areas. During supine ventricular rhythms septal P-R is shorter than P-R of indirect leads and septal activity precedes that of indirect leads. During block, with ventricular pacemakers, septal activity usually follows that of indirect leads. Records of one experiment with block are available where on lead 2 two ventricular pacemakers are shown but only one of these registers on the septal electrodes. These electrocardiograms from deep basal septal contacts probably show depolarization of the bundle of His, certainly show altered time relations when the pacemaker is ventricular, and suggest that not all ventricular premature beats spread to every part of ventricular muscle.

**Blood, plasma and extracellular fluid volumes in experimental low colonic obstruction.** A. H. TUTTLE (introduced by R. R. OVIKIAN, *Department of Physiology, University of Tennessee, College of Medicine, Memphis, Tennessee*). Although previous investigators (Roberts and Crundall, *Arch. Int. Med.* 50:150, 1932) and most standard texts state that dehydration is an important factor in low colonic obstruction, modern methods of body fluid measurement have not been applied in this condition. Consequently, experiments have been performed using such methods to determine the nature and magnitude of body fluid changes in low colonic obstruction. Following complete experimental obstruction of the sigmoid colon in 9 dogs, simultaneous plasma volumes by the T-1824 dye method and "extracellular" fluid volumes by the thiocyanate dilution method were determined. Between the 16th and 24th post-operative days, the following average reductions in total volumes were found: plasma 6%, blood (calculated) 14%, extracellular fluid 16%, and erythrocyte mass (calculated) 25%. However, relative to changes in body weight, each of the above values with the exception of the erythrocyte mass was found to be increased. Since the extent to which the animals lost weight exceeded the extent of water loss, it is apparent that the primary weight reduction following low colonic obstruction is due to loss of tissue with low water content, presumably fat. More severe dehydration has been demonstrated in dogs during starvation, yet such reduction in body fluid volumes were not fatal, and the animals recovered when allowed food and water. We conclude that circulatory embarrassment and/or extracellular dehydration is not a serious sequel of low colonic obstruction.

**The effect of low thiamine intake on the reaction time of women.** W. W. TUTTLE, MARJORIE WILSON

(by invitation) and KARL DAUM (by invitation) *Departments of Physiology and Nutrition, State University of Iowa, Iowa City*. A group of 12 women ate a standard diet containing all nutritional requirements except thiamine. The standard diet did not contain more than 0.14 mg thiamine per day. During a 6 week control period all the subjects ate the standard diet supplemented with adequate thiamine. During this period reaction times were measured so as to establish normal means for each subject. During a 6 week experimental period which followed, 6 subjects ate the supplemented diet, and 6 subjects ate the low thiamine diet. During this period measurements of reaction time were made. Reaction time to a light stimulus was measured with a Dunlap chronoscope. A comparison of the means shows that the reaction time of those eating low thiamine is significantly longer during the low thiamine period than during the period of adequate thiamine intake. The data also show that the reaction time of those on inadequate diet throughout the experiment did not become significantly longer. The data also support the conclusion that there was an increase in the dispersion of the accumulative frequency distribution of the reaction time of the subjects during the period that they were eating a diet low in thiamine.

**The effect of nicotine on spinal synaptic conduction.** A. VAN HARREVELD and GEORGE A. FEIGEN (by invitation) *California Institute of Technology, Pasadena, California*. In cats reflex action potentials were led off from the ventral roots of L7 or S1, while stimulating the homolateral dorsal root at the same segmental level. The injection of 5 mgm nicotine per kg bodyweight depressed or completely suppressed the monosynaptic spike, having a much smaller effect, which was usually temporary, on the polysynaptic activity. In agreement with this it was found that the kneejerk is suppressed, but that the flexion reflex is never abolished by such a dose of nicotine. Injecting more nicotine (up to 100 or even 200 mgm/kg) caused the return of a monosynaptic spike which often became larger than before administration of the drug. The polysynaptic activity also was considerably increased. In preparations in which the peripheral curare like effect of nicotine was prevented by establishing a separate circulation in the hind leg, it could be shown that the monosynaptic activity observed after large doses of nicotine is the equivalent of a myotatic reflex activity (kneejerk) like in the unpoisoned state. Nicotine, therefore, does not seem to have the blocking effect on synaptic transmission in the spinal cord which it shows in sympathetic ganglia and in striated muscle. The depressing effect of the drug in small amounts on the kneejerk is probably due to a side action.

**Effect of acclimatization on blood sugar re-**



**sponse to anoxia** EDWARD J. VAN LIERE, J. CLIFFORD STICKNEY and DAVID W. NORTUP. *Dept. of Physiology, School of Medicine, West Virginia University, Morgantown*. Control dogs fasted for 24 hours were subjected to anoxic anoxia (254 mm Hg) in a low pressure chamber and blood sugar determinations made at the end of 15 minutes. Several such determinations were made at weekly intervals. After the control figure had been established, the animals were subjected to intermittent anoxia (303 mm Hg) by keeping them in a low-pressure chamber four hours a day. Three of the four dogs showed acclimatization after a period of about 15 days since their blood sugar showed no significant elevation when exposed to a pressure of 226 mm Hg for 15 minutes. One animal which was extremely sensitive to anoxia showed only partial acclimatization. To demonstrate that there actually is an acclimatization for every altitude and to show that the adrenal glands were not exhausted the acclimatized animals were subjected to a pressure of 208 mm Hg for 15 minutes. Three of the four dogs showed a significant rise in blood sugar. The animal which showed only partial acclimatization died from anoxia. Deacclimatization was studied in the three remaining dogs. At weekly intervals they were subjected to a pressure of 254 mm Hg and the blood sugar determined at the end of 15 minutes. One animal had lost most of his acclimatization after about 15 days, another started losing its acclimatization after about six weeks, another is still acclimatized although five months have elapsed since he was exposed to intermittent anoxia.

**The role of the adrenal in the arterial pressure responses to severe hypoxemia.** A. VAN LOO (by invitation), A. SURTSIN (by invitation) and L. N. KATZ. *From the Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Illinois*. We have previously noted the effect of severe hypoxemia on the blood pressure of the dog. Breathing of pure nitrogen in open chested dogs causes the development of an acute severe hypoxemia with a primary pressor phase, followed in a minute or so by a progressively falling blood pressure. When air breathing is resumed, the blood pressure rises rapidly to greater than control levels (posthypoxemic rise). We were interested in determining the factors responsible for these pressor responses, during the hypoxemic phase and in the posthypoxemic phase. In unilaterally adrenalectomized animals, diversion into a syringe of the venous blood draining from the remaining adrenal resulted in no significant change in the hypoxemic pressor phase, but diminished considerably the posthypoxemic response. Blood collected in the phase of falling blood pressure during nitrogen breathing had markedly more pressor activity than that collected during the earlier phases of

hypoxemia. Re-injection of such collected blood immediately after the peak of the posthypoxemic response due to extra-adrenal factors caused the pressure to rise to a level similar to that attained in the control experiment. It is concluded that the adrenal gland plays little part in the production of the hypoxemic pressor response, but plays a major role in the production of the arterial pressor response after re-aeration. Pressor material liberated from the adrenal during severe hypoxemia does not exert its pressor effect until the tissues are re-oxygenated.

**Post-fracture nitrogen loss with and without methionine supplements to good and poor protein diets.** L. VAN MIDDLESWORTH and D. H. COPP (introduced by J. P. QUIGLEY). *Division of Physiology, University of California, Berkeley, and University of Tennessee, Memphis*. The effect of fracture upon daily urea + ammonia excretion was determined upon 6 groups of rats (6 animals per group) over periods of 20-90 days. The effects of the following synthetic dietary regimes were studied: (I) Complete diet containing 20% fibrin as the only protein; (II) Same as (I) but enriched with 2% dl-methionine; (III) Containing 10% casein as the only protein, otherwise complete; (IV) 9% casein plus 2% dl-methionine, otherwise complete (same nitrogen content as III). Young adult female rats, which had stopped growing, were used with (I) and (II). Young adult males were used with (III) and (IV). The daily dietary intakes (9 gm of diet/day) were limited such as to prevent growth. The animals weighed 175-200 gm and their weights were maintained relatively constant over a 30 day control period. After single femur fractures the rats on (I) and (II) showed a cumulative loss of 200-400 mg of nitrogen (above their basal excretion) during the first 8-10 days, after which their excretion returned to basal levels. The cumulative urea +  $\text{NH}_3$  loss of animals on diets (III) and (IV) was 200-280 mg of nitrogen but this required 24-28 days. Sham operations on (III) and (IV) resulted in the cumulative loss of only 20-60 mg of nitrogen and the increased excretion was not observed after 6-8 days. No consistent effect of methionine was demonstrated.

**Vitamin A deficiency in pregnancy.** G. VAN WAGENEN. *Dept. of Obstetrics and Gynecology, Yale School of Medicine, New Haven, Conn.* Normal pregnant monkeys and pregnant monkeys which had been less than a year on a diet deficient in Vitamin A were studied. The desquamation of keratinized epithelial cells as recorded in percentage of sediment in the vaginal lavage (method of Hartman) indicates ovarian activity. In young monkeys, before menarche, lavage yields few cells, while during the menstrual cycle vaginal contents may reach 50 to 100% density reflecting estrogen stimulation, then dropping at the

cycle end. During early pregnancy in the normal monkey desquamation remains at high levels as the many keratinized layers of superficial cells are shed just before, during and immediately after "implantation bleeding." In this first trimester the epithelium is reduced to a twentieth of its original thickness and appears as an inactive mucous membrane only a few cells thick. During the latter two-thirds of pregnancy, fluid from the vagina is clear and cell content extremely low. Paralleling this change in epithelial proliferation is the diminishing activity of the ovary.

In the A-deficient monkey keratinization of the vaginal epithelium persisted throughout pregnancy. This modification from the normal represents the imposed effect of vitamin A depletion common to many body epithelia and has been described in the pregnant rat by Mason ('35). The epithelial change here described in the monkey is probably an early digression from the normal at a low level of vitamin deficiency because it was compatible with the production of viable young showing no external developmental changes such as Warkany and Schraffenberger ('41) described in the rat.

**Fractionation studies of enterogastrone activity in pyloric ligation rats.** FRANK VISSCHER *From the Research Laboratories of The Upjohn Company, Kalamazoo, Michigan.* We have submitted preparations of enterogastrone to electrophoretic separation at pH 7.5 and have tested the resultant fractions for anti-secretory activity in pyloric ligation rats, using light barbiturate anesthesia and a two hour collection period. Seven fractions were each tested at three or more dosage levels to determine, by interpolation, the dose required for 50 per cent inhibition of volume of secretion. The fractions in order of increasing mobility gave the following approximate dosage requirements in mg per kg of rat: 6, 12, 23, 20, 18, 30, and 35. The most mobile fraction showed no inhibition at 20 mg per kg. The original material had a dosage requirement of 10 to 15 mg per kg.

We have also performed dialysis experiments involving three changes of external solution over a 72 hour period. About 50% of the material and nearly all of the remaining activity was recovered from the external solution. This material had an anti-secretory activity fully equal to that of the starting material, while the soluble fraction of non-dialysable material was nearly inactive.

**The capacity changes in the pulmonary vascular bed with the respiratory cycle.** MAURICE B. VISSCHER *University of Minnesota.* Experiments were performed with isolated lungs from heparinized dogs to determine pulmonary vascular bed volume. Cannulae were fixed in the pulmonary artery and left atrium respectively, connected to calibrated tubes acting as recording reservoirs. The levels of fluid in the calibrated tubes were recorded by

motion picture photography. The lung, cannulae and volume recorders were placed in a glass chamber, the tracheal tube was led out of the chamber and a second outlet was provided to alter the "intrathoracic" pressure. This system differs from those used previously by others, in that the head of pressure on the blood in the pulmonary vessels is always exposed to "intrathoracic" pressure. The earlier workers exposed the blood reservoirs to atmospheric pressure. Using the physiologically correct pressure head as described above entirely different results are obtained from those described by previous workers. It is found that the maximum blood capacity of the pulmonary vessels occurs at an "intrathoracic" pressure of  $-10$  cm  $H_2O$ . At more negative pressures the capacity of the lung vessels decreases progressively. For example, at  $-20$  cm  $H_2O$  in a 15 kg dog with lungs weighing 108 gms containing 50 cc of expressible blood the decrease in lung vascular capacity was 2.3 cc. When the pulmonary vessel reservoirs are exposed to the same pressures as those acting on the lungs it is found that changes in "intrathoracic" pressure in the ranges of normal inspiration and passive expiration result in decreases in lung vessel capacity during inspiration, proportional to the degree of lung inflation, and increases during expiration.

**Pupil dilatation in darkness as effected by intensity and duration of preexposure to white light.** IRVING H. WACMAN *Department of Physiology, Jefferson Medical College, Philadelphia.* The course of pupillary dilatation in total darkness was determined for humans and rabbits following an exposure of two minutes duration to different brightnesses (1.6, 16, 52 footlamberts) of white light and following varying durations (15 seconds to 3 minutes) of exposures of a constant brightness. For the human, the visual field subtended an angle of  $17^\circ$  fixated centrally. The measurements were made by means of infrared photography. After the stimulus light is turned off, the pupil dilates rapidly for the first twenty seconds at which point it has reached more than half its maximum diameter. It dilates slowly from then on until it reaches its maximum size in about 6 to 10 minutes. The initial point of the pupillary dilatation curve and as a result, its subsequent course are determined by the intensity of preexposure. After exposure to a high intensity the pupil is smaller than after exposure to a lower intensity and therefore, the curve describing its course of dilatation is displaced to the right of the latter on the time axis. The curves, as far as can be determined are parallel. Varying the length of exposure of a preadapting light of constant intensity does not displace the curve. Such curves tend to follow similar courses. The course of pupillary dilatation seems to be determined, therefore, by the size of the pupil immediately after the light stimulus is removed.

The effects of percutaneous stimulation on the

**circulation in normal and in paralyzed extremities**  
 K G WAKIM, J C TERRIER (by invitation) and  
 E C ELKINS (by invitation) *Rochester, Minne-  
 sota* After control values for blood flow were es-  
 tablished, the muscles in normal and in paralyzed  
 extremities were stimulated percutaneously by the  
 use of repeated condenser discharges at a frequency  
 of about seventy stimuli per minute for a period  
 of fifteen minutes. Determinations of blood flow  
 were again taken at the end of the period of stimula-  
 tion. The venous occlusion plethysmograph with a  
 compensating spirometer recorder was used for  
 the measurement of blood flow. Before and after  
 stimulation of the extremities, skin temperatures  
 were recorded galvanometrically. After electric  
 stimulation of the spastic lower extremities of  
 seven subjects, the blood flow in the stimulated  
 extremities consistently showed an increase (in  
 every one of the twenty two experiments) which  
 averaged 111 per cent over the control flow, with  
 a range of plus 32 per cent to plus 340 per cent. The  
 changes in skin temperatures were inconsistent,  
 namely, in twenty one experiments, the skin tem-  
 peratures were reduced in three and increased in  
 the rest. After stimulation, the spastic patients  
 experienced a reduction in their spasticity which  
 varied in magnitude and duration. Similarly, after  
 stimulation the blood flow in normal lower extren-  
 ities of eight healthy subjects consistently showed  
 an increase which averaged 86 per cent, with a  
 range of plus 10 per cent to plus 210 per cent. Nor-  
 mal subjects could not tolerate stimulation with  
 the same intensity as that applied to paralyzed  
 subjects. Of the eight subjects, six showed, after  
 stimulation, a reduction in the skin temperature  
 of the stimulated extremity, one showed no change,  
 and one showed an increase.

Sixteen experiments were performed on the flac-  
 cid lower extremities of seven subjects who had  
 lower motor neuron paralysis. After stimulation,  
 four patients showed a reduction in blood flow,  
 one showed no change, and the rest showed a slight  
 increase, the average was plus 17 per cent and the  
 range was minus 41 per cent to plus 48 per cent.  
 Before and after electric stimulation the skin tem-  
 peratures were recorded in 15 experiments, in nine  
 they were decreased after stimulation, and in six,  
 increased. The increase in blood flow occurring  
 after electric stimulation concomitant with reduc-  
 tion in skin temperature in spastic and in normal  
 extremities, and the insignificant increase in flow  
 in atrophic flaccid extremities after stimulation,  
 indicate that the increase in blood flow is con-  
 tributed chiefly by activated muscles.

**Interconversions of retinene and vitamin A *in  
 vitro*** GEORGE WARD *Biological Laboratories of  
 Harvard University, Cambridge* In intact retinas  
 the bleaching of rhodopsin by light liberates the  
 xanthene retinene, which is subsequently con-  
 verted to vitamin A. These processes can occur in

the complete absence of oxygen, and in tissues poi-  
 soned with iodoacetate. Suspensions of isolated  
 rod outer limbs, however, appear unable to convert  
 retinene to vitamin A. In 1942 we prepared a cell-  
 free brei from cattle retinas which accomplished  
 this conversion. These experiments were inter-  
 rupted by the War and have only now been re-  
 sumed. Dark adapted retinas can be lyophilized,  
 ground in a mortar, and extracted exhaustively in  
 the dark with petrol ether. The residue, stirred up  
 with neutral phosphate buffer, contains unaltered  
 rhodopsin. On irradiation this bleaches, and the  
 retinene formed is converted quantitatively to  
 vitamin A. Recently the reverse conversion of  
 vitamin A to retinene has been reported (Ball,  
 Goodwin and Morton, *Biochem J*, 40, Proc. ix,  
 1946). Vitamin A alcohol, let stand in the cold for  
 several days in contact with solid manganese diox-  
 ide, is replaced by a substance having a spectrum  
 and yielding an antimony chloride test like those  
 of retinene. We have confirmed these observations.  
 We find that the adsorption of vitamin A by man-  
 ganese dioxide plays an important part in the proc-  
 ess. Retinene is less strongly adsorbed, and as fast  
 as it forms it is displaced by new vitamin A. One  
 can perform the conversion within a few minutes  
 at room temperature, using a short column of man-  
 ganese dioxide, pouring a petrol ether solution of  
 vitamin A in at the top and drawing off a solution  
 of retinene in the filtrate.

**Further studies on the treatment and prophylaxis of experimental renal hypertension with renal extracts** G E WAKELIN, JOHN MARSHALL, HIROAKI MINATOYA, RUFUS WALKER and A KAPLAN *Department of Physiology, University of Illinois, College of Medicine, Chicago* In further work to determine the mechanism of the antihypertensive effect of hog renal extracts and to obtain a consistently potent, purified extract, we studied 2 new crude hog renal extracts in the treatment of renal hypertensive dogs and 5 fractions of one of the crude extracts in the prophylaxis of experimental renal hypertension in dogs. One of the crude extracts was prepared as previously described, except that autolysis was minimized by prompt acetone treatment at  $-20^{\circ}\text{C}$ . Daily intra-muscular injections of this extract for 10 months produced only a fair antihypertensive effect and high serum antirenin titres (10 and 16 units per cc) in 2 renal hypertensive dogs. Treatment with the second extract prepared from Viobin processed kidney powder for 5 months gave no antihypertensive effect and no antirenin in 2 hypertensive dogs and a fair effect and modest antirenin titre (2 units) in one animal. Five ammonium sulfate fractions were studied prophylactically. Only one dog was protected against chronic experimental renal hypertension, 8 animals developed chronic hypertension similar to 11 of the 14 controls, and 2 dogs developed renal insufficiency without hyper-

tension and without uterine cancerosis in contrast to 3 of the controls which died in typical malignant hypertension. The antirenin titres of the treated animals varied from 0.4 to 22 units, with 5 unit titres for the protected dog and the two animals showing fatal renal insufficiency without malignant hypertension.

The data do not permit conclusions relative to the renin antirenin mechanism as operative in the modest therapeutic and prophylactic effects observed. The results suggest that preliminary renal autolysis may be of significance in determining antihypertensive extract potency.

**The relationship between anticoagulants and lipemia** J. M. WALDRON (by invitation), and M. H. F. FRIEDMAN, *Department of Physiology, Jefferson Medical College, Philadelphia*. Hahn (1943) and Weld (1941) reported that a small dose of heparin intravenously cleared alimentary lipemia within five minutes and that this phenomenon did not occur in vitro. We found that heparin not only clears alimentary lipemia but also lipemia of carbon tetrachloride poisoning. Furthermore, other anticoagulants (a synthetic heparin like sulfonated polysaccharide and pontamine fast pink B L) have the same effect. In addition to clearing alimentary lipemia heparin has a reverse effect. If corn oil is administered in an amount which, in itself, does not produce lipemia, the blood will become lipemic within five minutes after the intravenous injection of heparin or the other anticoagulants. This lipemia-potentiating effect of anticoagulants does not occur in vitro or without prior administration of fat. Thus an attempt has been made to utilize this property of heparin as a test for intestinal absorption of fat. As used by previous authors and by us the term "lipemia" refers only to the turbidity of the serum or plasma without reference to the quantity or physical state of the fat. The turbidity returns to the heparin cleared serum if left in a test tube at room temperature for eighteen hours, whereas the lipemia induced by heparin does not clear under the same conditions. The occurrence of both the lipemia-clearing and lipemia-potentiating effects are correlated with blood coagulation time. The anticoagulant effect of heparin is modified by the ingestion of fat.

**Action potentials induced by indirect stimulation of rat muscle after adrenalectomy, KCl treatment and tetanus** SHEPPARD M. WALKER, *Dept. of Physiology, Washington University School of Medicine, St. Louis, Mo.* The records were obtained under ether anaesthesia from immature rats. Action potentials were recorded from the gastrocnemius muscle with a cathode ray oscillograph. Mechanical records of muscle contraction were made simultaneously using an isometric lever and optical recording. The cut sciatic nerve was stimulated with brief shocks 3 to 4 times threshold strength. The records from adrenalectomized ani-

mals were obtained after the appearance of signs of severe adrenal cortex insufficiency. Normal rats were injected intraperitoneally with 10 to 80 mgm of KCl per 100 grams of body weight. Tetanus was produced by repetitive shocks at the rate of 200 per sec. for 1 to 2 seconds. Control records were made from rested muscle of normal rats.

The mechanical responses to single stimuli were larger in muscle of adrenalectomized, KCl treated or tetanized rats than in muscle of control animals. The action potentials which accompanied the large mechanical responses showed no electrical activity which could be separated from the initial action potentials either when both lead electrodes were placed in the belly or when belly to tendon leads were employed. The duration of action potentials was longer in muscle after adrenalectomy, KCl treatment or tetanization than in control muscle. The height of action potentials was reduced by KCl treatment and by tetanization. The results indicate that the increase of mechanical response of adrenalectomized, KCl-treated and tetanized muscle to single stimuli is due to increased contractile strength of the muscle rather than to repetitive response of a part of the muscle fibers.

**Effect of secretin and pancreozymin on amylase and alkaline phosphatase of dog's pancreas** C. C. WANG (by invitation) and M. I. GROSSMAN, *Dept. of Clinical Science, University of Illinois College of Medicine, Chicago*. Highly purified secretin dissolved in saline was constantly injected by means of a perfusion pump into the femoral vein of anesthetized dogs with a cannula in the major pancreatic duct. Increase of flow was brought about by increasing the rate of injection of secretin. At least three or four samples of pancreatic juice were collected at each secretory rate level. Pancreozymin was injected in a single dose of 20 mg. during the response to a constant dose of secretin. All the samples were assayed for amylase and alkaline phosphatase content.

It was clearly shown that secretin had no stimulant effect on enzyme production, neither amylase nor alkaline phosphatase. As the volume of pancreatic juice was increased after increasing the rate of secretin injection, the concentrations of both of these enzymes were decreased, and the amounts of enzyme discharged per minute remained essentially unchanged in case of amylase and decreased in case of alkaline phosphatase. Pancreozymin, however, increased the concentration of amylase in the juice three or four-fold, but the alkaline phosphatase remained unchanged. This suggests a very significant point, namely, that the source of enzyme production is probably not the same for phosphatase as for the other enzymes in the pancreas. We propose that the ductule cells are responsible for the production of the phosphatase and the acinar cells for the other three enzymes, namely amylase, trypsinogen and lipase. Histo-

chemical demonstration of alkaline phosphatase in pancreatic tissue of the dog supports this proposal

**Serum accelerator globulin quantitative determination, purification and properties** ARNOLD G WARE (by invitation) and WALTER H SEEGER, *Department of Physiology, Wayne University, College of Medicine* Following the discovery of a factor in plasma which accelerates the interaction of prothrombin, thromboplastin, and calcium ions, we found that a similar factor is present in serum but it is far more potent in its accelerator action (Ware, Murphy and Seegers, *Science*, in press) We refer to the plasma factor by the term, plasma  $\Lambda$ c globulin, and to the serum factor by the term, serum  $\Lambda$ c globulin Thrombin produces serum  $\Lambda$ c globulin from plasma  $\Lambda$ c globulin, apparently by catalytic action Plasma  $\Lambda$ c globulin Thrombin Serum  $\Lambda$ c globulin Extremely small amounts of thrombin will produce the change Larger amounts of thrombin have a similar action but in addition actually destroy the accelerator activity The chemical properties of serum  $\Lambda$ c globulin are so similar to the properties of plasma  $\Lambda$ c globulin that the same method is used for purification as that described for the plasma accelerator factor (Ware and Seegers, *J Biol Chem*, in press) Serum  $\Lambda$ c globulin has been obtained in concentrated form both from serum and from defibrinated ovalated plasma It has also been obtained in high yields from defibrinated ovalated plasma after storage for 22 days at 5°C This fact and other data indicate that serum  $\Lambda$ c globulin is relatively stable in serum Quantitative measurements of its activity depend upon its ability to accelerate the interaction of prothrombin, thromboplastin, and calcium ions This is done by an adaptation of the 2 stage prothrombin analysis

**Influence of tea from leaves of *Ceanothus americanus* on blood pressure of hypertensive rats** HELENE WASTL, *Dept of Anatomy, Hahnemann Medical College, Philadelphia* Leaves of *Ceanothus americanus* (Rhamacea family) have been used widely as a satisfactory tea substitute during the American Revolutionary War The taste of this popularly called "New Jersey tea" is slightly more astringent In 1926 *Ceanothin*, alcoholic extract of the root bark from the shrub has been shown to have marked blood pressure and clotting time reducing properties The tea was tested on hypertensive rats, which were fed it once daily by hand

*Mm Hg systolic blood pressure*

|         | Normal  | Hypertension | New Jersey tea feeding | Cessation of tea feeding | Reduction of hypertension |
|---------|---------|--------------|------------------------|--------------------------|---------------------------|
| Average | 126     | 168          | 132                    | 168                      | -36                       |
| Range   | 105-140 | 125-230      | 103-163                | 125-230                  | -3 to -80                 |
| Average | 128     | 182          | 147                    | 185                      | -35                       |
| Range   | 110-140 | 154-230      | 119-171                | 160-230                  | -16 to -82                |

During tea-feeding-periods (first group, 18 rats, for 12 days, second group, 18 rats, for 24 days) reduction of hypertensive blood pressure increased in steps up to 6-8 days, when the final level characteristic for each individual rat is reached Lengthening of tea-feeding periods does not achieve better results The values refer to the whole pre-feeding, feeding and after-feeding periods averages, blood pressures were measured every 4 days G W Boericke, Dept of Therapeutics, Hahnemann Medical College, observed recently in a number of hospitalized cases of essential hypertension also encouraging reductions of blood pressure levels under New Jersey tea medication This study, on animals as well as on patients, will be continued

**Regeneration of insulin fibrils with several reagents and the nature of the inter-insulin bond** DAVID F WAUGH, M JANETTE SMITH (by invitation), and DARTHEA F FEARING (by invitation) *Dept of Biol, Mass Inst of Tech, Cambridge* Insulin fibrils (Waugh, *J Am Chem Soc* 66, 663, 1944) may be regenerated yielding products resembling insulin in crystallization, biological activity, and fibril formation Some compounds producing regeneration are (1) Acids 40% sulphuric acid at minus 10°C, and 35% hydrochloric acid at plus 5°C give crystalline yields of 50% (2) Alkalis 0°C to 15°C, 0.03N, and times of 5 to 10 minutes give 90% yields (3) Liquid ammonia minus 70°C yields 90-95% (4) Phenol regenerates over wide ranges of temperature and concentration giving maximum yields above 90% Several compounds such as nitrobenzene, chlorobenzene, and pyridine do not regenerate fibrils themselves and inhibit regeneration with phenol After treatment with such compounds fibrils may be recovered and subsequently regenerated with phenol, thus showing that they do not regenerate and destroy the regeneration product Salts, i.e. 0.1N sodium chloride and sodium acetate, inhibit regeneration with those compounds tested (2 and 4) indicating that repulsive forces between similarly charged particles are important This agrees with the inability of phenol to regenerate over a 3 pH unit range near the isoelectric point At 60°C and fibril concentration of 0.4%, 10-12% phenol regenerates rapidly In 5% phenol fibrils will elongate in the presence of native insulin An attempt will be made to show that these observations support a conclusion drawn previously (loc cit) that the inter insulin bond is due to the coalescence of hydrophobic residues

**Leucocytosis of guinea pigs deficient in the anti-stiffness factor** VIRGINIA WEINER (by invitation) and ROSALIND WELZEN, *Dept of Zoology, Oregon State College* Guinea pigs fed a skim milk diet, supplemented by the known vitamins and necessary minerals but deficient in the anti stiffness factor, developed a characteristic wrist stiffness Autopsies showed the muscles to be finely streaked with calcium deposits running parallel to the

muscle fibers. Large deposits of tricalcium phosphate were frequently found along the body wall, around the joints, and in numerous organs (Wulzen *et al*, *Am J Physiol* 133, 500, 1911). Leucocyte enumerations were made on 26 guinea pigs which had been fed for 15 to 98 weeks on the diet deficient in the anti-stiffness factor. These animals had white counts of 11,000 to 30,000 (mean 17,668). Fifty-five young and adult male and female guinea pigs on a grain and kale diet had leucocyte counts of 1,000 to 11,900 (mean, 7,459). The "Student's" *t* test was used to demonstrate the difference in the means of the two groups. The *t* was  $-11.33$ , degrees of freedom 79. These results indicate that deficiency of the anti-stiffness factor in guinea pigs produces a significant leucocytosis.

**The effect of serum hypersensitivity reactions upon the electrocardiogram.** GRACE L. WIKTENBERGLER and ROBERT A. HAFKESBRINC. *Department of Physiology, Woman's Medical College of Pennsylvania, Philadelphia, Pennsylvania.* During an investigation of the effects of the sulfonamides upon the myocardium, electrocardiographic changes indicating alterations in the physiologic state of the muscle as shown by prolonged conduction time, ST segment deviation and change in the direction of the T wave were found in 15% of the animals. No electrocardiographic changes occurred with the initial course of the drug, even in animals whose controls showed prolonged conduction time, heart block and ST segment shifts indicating established cardiac pathology. The cardiac changes appeared with subsequent courses of the drug and, although transient in some and persistent in others, were always associated with severe drug reactions.

Since the side reactions to the sulfonamides suggest hypersensitization with the drug acting as the sensitizing antigen, the present study to compare the functional effects of serum sickness and sulfonamide hypersensitivity upon the myocardium was begun. In the first series of serum sensitized rabbits, the electrocardiogram showed only asphyxial changes in the animals dying of anaphylactic shock and no significant changes have as yet appeared in the three animals that survived the shocking dose. The second series is now being followed. In the series of serum sensitized dogs now being studied, changes have appeared in the electrocardiogram which cannot be attributed to asphyxia.

**The renal excretion of strong electrolytes.** LAURENCE G. WESSON, W. PARKER ANSLOW, JR. and HOMER W. SMITH. *New York University College of Medicine.* Indirect evidence (Smith, Oliver *et al*) indicates that roughly 85 per cent of the sodium and water in the glomerular filtrate are reabsorbed by the proximal tubule and thin limb in the mammalian kidney, and that tubular urine

is isosmotic with plasma half way down the proximal tubule (Oliver *et al*). Further quantities of sodium and water are reabsorbed in the distal tubule by independent processes. Observations on dogs during osmotic diuresis induced by mannitol reveal that up to 65 per cent of the water of the glomerular filtrate may be excreted with a simultaneous excretion of only 27 per cent of the filtered sodium. The sum of the osmotic constituents in the urine remains practically identical with the plasma. It is concluded first, that the proximal reabsorption of sodium and water are at least partially independent processes and second, that proximal water reabsorption is a process of passive diffusion initiated by the active reabsorption of sodium, with its attendant anions. Passive osmotic equilibration may be completed in the thin limb so that the urine delivered to the distal tubule is normally isosmotic with plasma. If the limiting values in either sodium or water reabsorption in the distal tubule can be determined experimentally, the partition of both constituents between proximal and distal reabsorption can be calculated.

Tentative evidence is adduced that under the action of ADH distal reabsorption of water reaches a maximal rate ( $T_{m_{H_2O}}^d$ ) and that under conditions conducive to stability the distal reabsorption of sodium is also limited by a maximal rate ( $T_{m_{Na}}^d$ ). Under such conditions sodium excretion is conditioned by the filtration rate as well as the plasma level of sodium.

**Effect of low sodium and Kempner diets on renal hemodynamics and electrolyte excretion in hypertensives.** RAYMOND E. WESTON, LEON HELLMAN (by invitation), DORIS J. W. ESCHER (by invitation), and LOUIS LEITER (by invitation). *Medical Division, Montefiore Hospital, New York 67, N. Y.* The Kempner rice diet, which has been recommended for the treatment of hypertension, provides an opportunity to follow changes in renal hemodynamics and electrolyte excretion during severely restricted sodium and protein intake. Therefore, in selected hypertensive patients without demonstrable specific renal pathology, glomerular filtration rates, renal plasma flows, maximal tubular PAH excretory capacities, and sodium, chloride, and urea excretions were studied on normal diets, after several weeks on low sodium (0.8) but normal protein (65 grams) diets, and after four to sixteen weeks on the Kempner (0.2 grams sodium and 30 grams protein) regime.

Preliminary observations indicate that in hypertensives, the low salt diet produces a decrease in filtration fraction, since there is a significant decrease in GFR with a lesser change in RPF, and no change in  $T_{m_{PAH}}$ . In some patients, the more severe sodium restriction and/or the reduced pro-

tein of the rice diet result in a further decrease in GFR, a greater decrease in RPF, and some diminution in  $T_{\text{PAH}}$ . As the diets were varied, the changes observed in electrolyte excretion were as expected, with certain exceptions which apparently are characteristic of the hypertensive kidney. In this small series, none of the patients exhibited a significant drop in blood pressure while on either the low salt or the rice diet.

Further experimental analysis of these results is in progress, with particular emphasis on the influence of plasma volume, extracellular fluid volume, electrolyte and protein metabolism, and endocrine factors.

**Effects of exercise on renal circulation.** H. L. WHITE and DORIS ROLF (by invitation) *Dept. of Physiology, Washington University School of Medicine, St. Louis, Mo.* The effects of exercise on para aminohippurate (PAH) and inulin clearances (subcutaneous administration), on venous plasma glucose, mean blood pressure (diastolic plus 40% pulse pressure), renal vascular resistance, and urine protein were determined in six normal human males. Blood pressures were not obtained during exercise. Control periods were during recumbency. Light exercise (walking alternating with slow running for 15 minutes, or slow running for 12 minutes) produced a slight fall or no change in PAH clearance, with slight further falls in first two post-exercise periods; there was no significant change in filtration fraction.

Heavy exercise, to exhaustion, panting and profuse sweating, by running at maximum capacity for 12 minutes, gave great falls (to 20% of normal) in PAH and inulin clearances during exercise, the values had not returned to normal within 60 minutes. Filtration fractions showed no consistent changes during and after exercise. Renal vascular resistance increased at least 5 fold (taking mean arterial pressure during exercise as normal) during exercise and was still somewhat above normal an hour later. All urines were protein free before and during heavy exercise, but urines formed during first and second post exercise periods contained protein, which had disappeared by the third 20 minute post exercise collection. This supports but does not prove the view that during heavy exercise many glomeruli may have no blood flow. Rise in plasma glucose, probably indicative of adrenalin output, was slight or absent on light but up to 100 mgm % on heavy exercise.

**Circulatory responses to exposure to barometric pressure of 30 mm Hg.** WILLIAM V. WHITEHORN, *Dept. of Physiology, Ohio State Univ.* Nembutal anesthetized dogs explosively decompressed from  $560 \pm 5$  to  $30 \pm 5$  mm Hg show drops in arterial pressures initially similar to those seen following explosive decompression to less extreme pressures.

Recovery to normal levels during exposure to the reduced pressure does not occur, however—mean pressures falling to an average level of 70-7 mm Hg 30 sec after decompression and remaining essentially unchanged for periods up to one minute. Arterial pressure curves indicate marked diminution in pulse pressures or actual disappearance of arterial pulses within 30 seconds after decompression although e.g. records show continuing electrical cardiac activity. Observations on exposed arteries and veins under these conditions reveal massive venous but absent arterial intravascular bubble formation. The described phenomena do not occur with decompressions to final pressures greater than about 50 mm Hg. It is postulated that the circulatory changes described are the result of blocking of veins and pulmonary vessels by gas formation with resulting reduction or absence of left ventricular output. The maintenance of mean arterial pressure at a constant low level under these conditions is not entirely clear but may be the result of increased tissue pressures due to interstitial gas formation at final pressures less than 47 mm Hg.

**Rhesus hyperkinesia by subthalamic lesion.** JOHN R. WHITTIER introduced by FRED A. METTLER *Department of Neurology, Columbia University, College of Physicians and Surgeons (Motion Picture).* A variety of abnormal involuntary movements have been produced in rhesus monkeys by small electrolytic lesions placed in the region of the subthalamic nucleus with stereotaxic technique. Choreoid, athetoid, and choreo-athetoid patterns appeared upon recovery from anesthetic and persisted in most cases until sacrifice, as long as two months after operation. They varied among the animals from the appearance of irregularly alternating movements at a single joint to such forceful sequences in axial and appendicular muscles that hips and limbs were flung about in a violent manner and the act of feeding became a hectic conflict between voluntary and involuntary activity. Unequivocal hyperkinesia appeared in 20 monkeys of a series of 80, and in every instance the subthalamic nucleus was involved by the lesion. In 37 animals for which histological preparations are available to date the subthalamic nucleus was damaged in 11 who showed no hyperkinesia. Tentative analysis suggests that in these cases the involvement of the nucleus in the lesion was small, or extension by the lesion into surrounding structures was considerable.

Lantern slides illustrate the lesions and a motion picture demonstrates varieties of hyperkinesia obtained.

**Preparation of anti-ulcer substance from bovine urine.** ARNE N. WICK and FRANCES PAULS (introduced by ELYON M. MACHAY) *Scrrips Metabolic*

*Clinic, La Jolla, Calif* The preparation of an anti-ulcer factor from human urine by charcoal adsorption has previously been reported (Wick, Irish, Pauls, and MacKay, *Proc Soc Exper Biol and Med*, 64: 10, 1917) Examination of cow urine (obtained from milking herds 75 per cent of which are customarily bred) for this factor disclosed an excellent source for a non-toxic anti-ulcer preparation This material can be obtained by treating the urine with carbon followed by elution of the active principle with aqueous acetone Crude dialyzed samples obtained by this method completely prevented gastric ulceration in the rat assay (Pauls, Wick, and MacKay, *Gastroenterology*, 8: 774, 1947) when administered intravenously at a dose level of 100 mg per kilogram Chorionic gonadotropic assays in the immature rat give a negative response at 1 IU per mg of test sample The benzoic acid procedure (Gray, Wiczorowski, Wells, and Harris, *Endocrinology*, 30: 129, 1912) when applied to bovine urine in contrast to human urine, is not suitable for the isolation of the ulcer factor (urogastrone) because of the apparent presence of a solubilizing agent

**The effect of body temperature on the respiratory transformation of intravenous carbohydrates in amytal-anesthetized dogs** M WILKUCHOWSKI (introduced by WILLIAM H CHAMBERS) *Physiological Institute of the Medical Faculty, University of Lodz, Poland* Moderately deep amytal anesthesia was maintained in dogs during continuous infusion of sugars at constant rate of 40 g /sq m /hr, with rectal temperature varying from 33.7-41.8°C Total utilization of carbohydrate, as well as ventilation and oxidation, increased with the rise of body temperature, the ratio of ventilation/O<sub>2</sub> consumption and heat production above basal grew constantly Specific dynamic action of glucose, fructose, maltose and galactose, referred to their total utilization, increased with body temperature according to a straight line equation The curves run parallel and fairly closely to each other (e.g., for glucose, 4% at 35°C, 13% at 40°C) similar to a normal range to those of unanesthetized dogs Intravenous glucose at rates of 30-214 g /sq m /hr was supplied to amytalized dogs for various ranges of body temperature When plotted against blood glucose concentration, similar curves were obtained for total utilization, ventilation, O<sub>2</sub> consumption, CO<sub>2</sub> elimination and heat production The curves rose at an exponential rate to a peak value which remained constant up to 4000 mg % of blood glucose The peak value was about 1000 mg % of blood glucose when the body temperature fell within normal limits In hypopyrexia the peak was lower than 1000 mg % and in hyperpyrexia it was higher At the peak value glucose oxidation was  $\frac{1}{2}$  of total assimilation, as in the unanesthetized animal (e.g., above 39°C, with a blood sugar of 1200 mg %, 20

g/sq m /hr were oxidized out of 100 g assimilated) Amytal anesthesia does not seem to effect carbohydrate transformation except when changes occur in body temperature Body temperature appears to be a factor of great importance in the consideration of the specific dynamic action of intravenous carbohydrates

**Glucose metabolism in marine invertebrates** CHARLES G WHEELER *The Biological Laboratory, Fordham University, New York* There is practically no information about metabolism in marine annelids to be found in the literature Some studies have been made on the effect of starvation on the lipids in *Phascolosoma* (*J Cell and Comp Physiol*, 29: 179, 1917) A series of experiments was, consequently, made to ascertain the effect of increased temperature on glucose metabolism in the annelid, *Phascolosoma gouldii* Body fluid was removed from 12 specimens, which had been kept at 20°C for several days, and analyzed for glucose Using the Iolin method an average of 17.3 mg glucose/100 cc fluid was found Using the Somogyi filtrate 16 mg /100 cc glucose was found A second dozen phascolosomas were put into a salt water bath at 30°C for 30 minutes after which the body fluid was analyzed With the I method the glucose value was 30.2 mg /100 cc, with the S filtrate, 26.1 mg /100 cc The differences are statistically significant Apparently, the increased temperature stimulated the breakdown of glycogen, stored in the muscles or elsewhere, to form glucose The ultimate fate of the large amount of glucose is now being studied with the view to throwing some light on the carbohydrate metabolism of marine invertebrate animals

**Relationship in the dog between inferior vena caval pressure and total plasma protein** J LARUE WILLY, MICHAEL NEWTON and J B TRACY, JR, (introduced by H C BAZETT) *Department of Physiology, Medical School, University of Pennsylvania, Philadelphia, Pa* Utilizing changes in the ratio Total Hb/Total Protein = Hb concentration/Plasma protein concentration (1 - hematocrit) as indicating fluctuations in total plasma protein, (Speelman, Newton and Post, *Am J Physiol* 150: 628, Oct 1917) it was found in human subjects that total circulating plasma protein increased over periods of several hours when posture was changed from recumbent to erect The effect on the ratio Hb/Protein, of artificially increasing venous pressures below the heart was studied in acute experiments on dogs All animals were first splenectomized to eliminate at least one variable reservoir of erythrocytes The inferior vena cava was constricted either between the diaphragm and heart, or between the renal veins and liver, resulting in elevation of distal caval pressure by 100-200 per cent Control animals underwent the same operative procedures but had no constriction ap



plied to the vena cava. Results in 7 experimental animals compared to 8 controls were inconclusive. There was a slight downward trend in the ratio Hb/protein when the thoracic inferior vena was constricted. However total circulating hemoglobin may have changed somewhat under conditions of these experiments, and the magnitude of changes in ratio do not justify the assumption of changes in total protein. Chronic preparations now being studied may yield more positive results.

**The systolic arterial pressure gradient as a measure of local peripheral resistance.** ARNOLD H. WILLIAMS (by invitation) and HENRY A. SCHROEDER, *Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri.* The hemodynamics of any artery may be simplified and likened to an expansile reservoir in which the pressure is principally regulated by the relative rates of cardiac input and arteriolar drainage. When the input is suddenly checked, as in diastole, the intraarterial pressure is maintained in part by the elastic recoil of the arterial wall but to a much greater extent by the rate of outflow into the capillary bed. The outflow rate depends on the caliber of the arteriolar "stopcock" which is the principal site of peripheral resistance. It follows that the gradient of blood pressure fall, distal to the point of a sudden occlusion of an artery, is a measure of the outflow or peripheral resistance, and that the arterial pressure approximates the local venous pressure with prolonged occlusion. This principle has been employed in dogs and man as a measure of peripheral resistance in various vascular areas. After arterial occlusion at the peak of systole the subsequent intraarterial pressure gradient has been determined with the Hamilton manometer in various end arteries such as those supplying the limbs, the mesentery, and the kidney. It is probable that this technique can not be utilized as a quantitative measure of peripheral resistance. However, it is extremely useful in following the duration and site of local vasoconstrictor action after the injection of pressor and depressor agents. The changes that occur are exaggerated by logarithmic plotting of the pressure gradients. Hemodynamic analyses of the factors involved and mathematical analyses of the gradients obtained are in progress.

**Sodium, potassium and chloride in leucocytes.** D. L. WILSON (by invitation) and J. F. MANERY, *Department of Biochemistry, University of Toronto, Toronto, Canada.* Rabbit leucocytes were equilibrated in Ringer-Dale solutions which contained concentrations of chloride varying from 25 to 160 m eq per litre, by substituting appropriate amounts of sodium sulphate for sodium chloride the solutions were made isosmotic. Suitable tests showed the leucocytes to be viable in all solutions

used. Packed cells, obtained by centrifugation, were analysed for sodium, potassium, chloride and water. The data indicated that intracellular chloride concentrations varied directly with those outside, always being lower at external concentrations above 55 m eq per litre. Evidence of an anion exchange was obtained. Although the analytical methods used for the determination of sodium and potassium gave results reproducible to  $\pm 1\%$ , the intracellular concentrations varied over a range of  $\pm 20\%$ . Under any set of conditions, however, the sum of the potassium and sodium concentrations remained constant suggesting that a reciprocal relation existed between the two cations. After aerating gently for 45 minutes in a Ringer-Dale solution containing 115 m eq of chloride per litre ( $37^\circ\text{C}$ ), the leucocytes contained 80% water, 80 to 90 m eq of chloride, 65 to 100 m eq of sodium and 75 to 110 m eq of potassium per l of cell water. These figures imply that factors other than the external concentration of sodium and potassium influence the concentration of these cations within the cells.

**Comparative properties of six phenethylamines.** CLAUDE V. WINDER, MONA M. ANDERSON (by invitation) and HERVEY C. PARKE (by invitation), *Research Laboratories, Parke, Davis & Co., Detroit, Michigan.* The hydrochlorides of phenethylamine, its  $\alpha$ - and  $\beta$  methylated derivatives, and the three corresponding N-methyl compounds (racemic forms in all cases of asymmetry) were studied along with l-ephedrine in phenobarbitalized, atropinized dogs. Average molecular pressor potencies (46-500  $\mu\text{gm}/\text{kgm}$  dose level) ranged between a few and several thousandths of epinephrine's. The  $\beta$  methylated compounds were significantly less potent. Intravenous nasal paranasal decongestant action was recorded volumetrically (special miniature spirometer). Decongestant efficiency was defined as the ratio of decongestant potency to pressor potency as determined against respective epinephrine scales from a common set of injections—an efficiency index with epinephrine set at unity. The parent amine and its N-methyl derivative were least efficient (significantly less than epinephrine), the  $\beta$ -methylated intermediate, and the  $\alpha$  methylated most. In general, the primary amines were more efficient than the secondary. Inferences concerning nasal vasoconstrictor efficiency bear reservations. Durability of action was measured in terms of time from beginning of effect until recession to half value. Its ratio to the same measure of the equipressor epinephrine response was termed the 'duration index' and was satisfactorily independent of dose level. Tachyphylaxis was studied systematically by repeated injections at decreasing time intervals of ca. equipressor doses (174-571  $\mu\text{gm}/\text{kgm}$ ). The non-chain methylated amines were least, the J-methylated intermediate, and the

$\alpha$ -methylated most durable and tachyphylactic. *l*-Ephedrine was somewhat less durable and less precipitously tachyphylactic than the non hydroxylated isopropylamines.

**On the nature of tachyphylaxis and related phenomena** CLAUDE V. WINDER and MONA M. ANDERSON (by invitation) *Research Laboratories, Parke, Davis & Co., Detroit, Michigan*. Tachyphylaxis of *dl* amphetamine, *dl* desoxyephedrine and *l* ephedrine hydrochlorides, at *ca* equipressor dose levels, was studied in arterial pressure and nasal (de) congestion (calibrated volume recorder) of phenobarbitalized, atropinized, vagotomized dogs. In each experiment one drug was repeatedly injected at intervals decreasing from 1 hr to 10 min. Tachyphylaxis in pressor and decongestion effects were strikingly parallel. While blood pressure more or less completely returned to 'normal' between injections, successive decongestions of the neurally isolated mucosa were relatively well-sustained and correspondingly additive. Plots of cumulative peak decongestion, as percent of 'total possible decongestion' on a probability scale, against logarithm of cumulative dose, yielded amazingly satisfactory linear fits in all cases. Hypotheses met by such plots are: (a) the dynamic proportion of a population of 'receptor points' occupied by the agent is of the nature of a statistical (probability) cumulation according to the agent's concentration, (b) the measured end effect is analogous to the pyramiding of successive orders of statistical samples between initial physico-chemical event and final functional change, (c) the statistical distribution is log-normal with respect to the agent's concentration, (d) a series of tachyphylactic responses reflect successive (more or less overlapping) stages of cumulative probability of receptor occupation, (e) the cumulating factor (in equal arithmetic, diminishing logarithmic steps) may be more (blood pressure) or less (mucosa) compensated between administration. Computations predict that tachyphylaxis, potentiation, and certain passive 'interferences' may be in important degrees kaleidoscopic manifestations of (a) compactness of probability range of agent-receptor engagement, (b) dose, (c) disposal, and (d) number of previous injections.

**Gastrointestinal and hematological responses in dogs to large doses of histamine and an antihistaminic drug** CHARLES A. WINTER and CHARLES W. MUSHETT (by invitation) *Merck Institute for Therapeutic Research, Rahway, N. J.* Previous workers have produced experimental peptic ulcers in dogs by prolonged histamine administration. However, when an animal is protected against the acute lethal effect of histamine by an antihistaminic agent, relatively large doses of histamine may be given, thus reducing to a few days the time required to produce ulcers. Antihistaminic drug

(Neo antergin) was administered 10 mg/kg in aqueous solution subcutaneously once half hour before histamine, followed by another dose intramuscularly in oil at the time of histamine administration. Each of 3 dogs received a single injection of histamine in oil beeswax, 20 to 40 mg/kg intramuscularly. Sacrifice 18 hours later revealed denudation or discrete ulceration in the duodenum in all 3. Typical ulceration was seen in 8 dogs receiving 2 to 5 such doses. In this group, sacrificed 3 to 7 days after the first dose, all animals had duodenal ulcers, and 7 had gastric ulcers.

Hemoconcentration occurred regularly within 2 to 4 hours following an initial injection of 40-60 mg/kg of histamine, and was still apparent, though decreased, after 24 hours. Initial leukopenia occurred within a few hours, but was sometimes masked by the coexisting hemoconcentration. However, within 24 hours a leukocytosis of variable degree usually developed, which often became more marked after additional histamine injections. Most animals thus showed a 2 to 4 fold increase in total leucocyte count. An increased erythrocyte sedimentation rate occurred frequently within 24 hours. These data indicate that the antihistaminic drug prevented neither the gastrointestinal lesions nor the hematological effects produced by histamine.

**The transport and excretion of uric acid in man**  
**IV. The renal mechanism for urate excretion**  
 W. Q. WOLFSON (by invitation) and R. L. VINE (from the Department of Biochemistry and the Department of Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago). A major obstacle in the study of human urate excretion has been the difficulty of provoking hyperuricemia in normal subjects without the toxicity accompanying intravenous administration of urate. This may be overcome by administering large fractional doses of nucleate orally on the day preceding the clearance. Administration terminates at midnight and clearances are determined on a falling curve. Urate levels up to 12.5 mg % were attained. At an average plasma urate of 6.26 mg %, the clearance represented 8.3% of the glomerular filtration rate. At 10.06 mg %, the urate clearance represented 9.5% of GFR. These data are incompatible with the current theory that urate only appears in human urine when a tubular reabsorptive mechanism of limited capacity is exceeded. An alternative, but improbable, hypothesis is that under normal conditions tubular reabsorptive mechanisms are not saturated but do not act rapidly enough to extract urate completely from the glomerular filtrate. From consideration of certain pharmacological data, and from the fact that only about 6% of the plasma urate passes into the CSF (J. Clin. Invest. 26:991, 1947), it appears probable that only a small fraction of the

plasma urate is ultrafiltrable through the human glomerulus. As only 20% to 30% of the plasma urate is bound to plasma protein in ultrafiltration experiments, the failure of most of the plasma urate to pass the human glomerulus appears not to result from protein binding. An alternative possibility is that the major portion of the plasma urate circulates as polymeric urate complexes. This state has been demonstrated to occur in the azotemic chicken (*Am J Physiol* in press).

**Anaphylactic shock in a restricted circulation system excluding the liver in the dog.** PARKER H. WOODARD (introduced by K. E. JOCHIM) *Dept of Physiology, Univ of Kansas, Lawrence.* Most previous work has supported the contention that it is necessary for the liver to remain in the circulation for the drop in blood pressure to occur in canine anaphylaxis. However, some recent work in other laboratories presents evidence contrary to this view. The experiments here presented indicate that not only is the liver unnecessary but that the fall in pressure occurs in a somewhat restricted circulation system.

Dogs were sensitized to horse serum and after the lapse of 12 to 14 days each animal was tested for sensitivity by determining the ability of its blood to passively sensitize normal dogs. If proven sensitive, each dog was anesthetized with nembutal intravenously and after opening the chest, the following vessels were ligated: the azygos, internal mammary and right subclavian veins, the right subclavian and internal mammary arteries, the vena cava above the diaphragm and between the liver and the diaphragm, and the aorta just distal to the left subclavian artery. Blood pressure was recorded from the left subclavian artery.

When a shock dose of horse serum was introduced intravenously into this preparation, the characteristic fall in blood pressure resulted. In the animals that were tested there was no change in blood coagulation time.

**Photoelectric determination of blood oxygen saturation in man.** E. H. WOOD, J. E. GERACI (by invitation) and D. L. GROOV (by invitation) *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota.* The Millikan oximeter cuppiece has been modified so that a transparent pneumatic pressure capsule is interposed between the light source and the ear. This cuppiece makes possible measurement of the red and infra red light transmission of the bloodless (pressurized) ear and of the normal heat flushed ear. The differences in light transmission between the bloodless and flushed ear are a measure of red and infra red transmission of the blood interposed in the optical path of the cuppiece. The ratio of the light transmission of blood in the red and infra red is a function of the oxygen saturation of this blood. The relationship between this ratio and arterial

oxygen saturation has been determined by comparing the results of Van Slyke analyses of 143 arterial blood samples from 37 individuals with the average results of instrumental readings obtained during the period of withdrawal of the samples. Normal white and negro subjects were used and patients with suspected arterial hypoxemia. Arterial samples were obtained during rest, exercise and while breathing gas mixtures of from 100 to 8 per cent oxygen content. The standard deviation of the differences of the individual photoelectric determinations from the mean calibration line was 2.4 percentage saturation points. On the same basis a photoelectric cuvette has been constructed in which the blood is contained in a 2 mm I.D. polythene tube (volume 0.5 cc). This device attached to a cardiac catheter has been used for immediate determination of the oxygen saturation of cardiac blood samples during diagnostic cardiac catheterizations.

**A technique for obtaining multiple arterial blood samples applied to the study of cyanosis in man.** (Kodachrome Motion Picture) E. H. WOOD, G. E. MONTGOMERY, JR. (by invitation) and J. E. GERACI (by invitation) *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota.* The sampling system which is coated internally with silicone and has a volume of 0.6 cc consists of an internally polished No. 20 needle, fitted into a glass adapter which is connected to a metal stopcock via a 15 centimeter length of 2 mm I.D. polythene tubing. The stopcock is adapted for connection to a hypodermic syringe. The syringe and sampling system are filled with sterile isotonic saline containing 20 mgm of heparin per liter. To facilitate the arterial puncture an armboard is used which fixes the hand in the position of extreme extension at the wrist. The site of the puncture (radial artery near the styloid process) is infiltrated with 1.0-2.0 cc of 2 per cent procaine hydrochloride. After the puncture is completed and the needle taped in position, arterial samples may be obtained at will by opening the stopcock. Approximately 1 cc of blood is discarded before beginning collection of each sample. The system is cleared of blood after each sampling period by reinjection of a few cubic centimeters of the sterile heparinized isotonic saline solution. This procedure has been carried out on 90 individuals. Approximately 500 arterial samples have been obtained from these subjects while at rest, breathing various oxygen mixtures, or walking on a power driven treadmill. The technique has been used to study the correlation of arterial oxygen saturation with clinical estimations of the degree of cyanosis induced in normal negro and white subjects by breathing low oxygen mixtures.

**The pattern of cutaneous representation in the rat's cerebral cortex.** CLINTON N. WOOLSEY and

**D H LEMESSURIER** (by invitation) *Dept of Physiology, Johns Hopkins University, School of Medicine, Baltimore 5, Md* Somatic areas I and II were mapped in the rat's cortex by the evoked potential method. Of particular interest is the simplicity of the pattern of representation in somatic area I (postcentral homologue). The pattern may be visualized by imagining a rat lying, right side up, on the left hemisphere with its tail near the midline and with the mid-dorsal line of the body from tail to nose extending across the hemisphere along the rostral border of the visual, auditory and somatic II areas. The leg, arm and face representations then, extend rostrward in the cortex so that the pattern presented is a somewhat distorted picture of a rat with its various parts related to one another in much the same way as in the actual animal. The results call for a re-examination of the details of tactile representation in other species so that the evolution of the pattern as seen in primates may be understood.

**Relation of retinal and optic nerve response to intensity of illumination of the grasshopper eye**

**VERNER JOHN WULFF** (by invitation) and **THEODORE LOUIS JAHN** *Department of Zoology and Physiology, University of Illinois, and Departments of Physiology and Zoology, State University of Iowa* Illumination of the eye of grasshoppers (*Melanoplus differentialis*) results in electrical responses in the visual system that can be recorded in the following temporal order: 1) the retinal action potential and 2) a slow negative variation on the surface of the nerve between the optic ganglion and the brain. The latter potential is presumably a sign of the asynchronous activity of neurones within the optic ganglion. The latency of these responses, measured from the beginning of the light stimulus, increases as the stimulating light intensity decreases. These relations are such that the time interval between the beginning of the retinal response and the beginning of the nerve response called the retinal-nerve interval also increases as the intensity of stimulation decreases. This regular increase in the retinal nerve interval is correlated with a reduction in magnitude of the retinal action potential and an increasing delay in the time of occurrence of the maximum of this action potential. In other words, the retinal nerve interval increases as the rate of change of potential across the retina decreases. This increase in the retinal-nerve interval supports the hypothesis that the retinal action potential is the process or a sign of the process which is responsible for the activation of nervous elements in the optic pathway.

**The effect of local graded pressure upon electrical activity and excitability of the motor cortex**

**J E ZIEGLER** and **T W RASMUSSEN** (introduced by **HERBERT JASPER**) *Department of Neurology and Neurosurgery, McGill University and the Mon-*

*trcal Neurological Institute* The electrical activity and excitability of the motor cortex was measured beneath a disc one half to 2 cm<sup>2</sup>, during the application of pressures between 20 and 70 grams. At a critical pressure per unit area, the electrical activity and excitability of the cortex beneath the disc disappeared without significant change in the cortex adjacent to the disc or from other portions of the ipsilateral or contralateral hemisphere. With a 0.5 cm<sup>2</sup> disc, the cortical excitability was suppressed within 30 to 60 seconds following the application of 30 grams of pressure. With a 1 cm<sup>2</sup> disc, the electrical excitability disappeared within 1 to 12 minutes (average 3.6 min.) at 50 to 60 grams pressure, and between 1 and 5 minutes (average 2.5 min.) with 70 grams pressure. The local electrical activity from bipolar recording electrodes beneath the 1 cm<sup>2</sup> disc disappeared with 70 grams of pressure for 1 to 5 minutes (average 2.6 min.). Local electrical activity tends to disappear in about the same time as does electrical excitability although several exceptions occurred with pressures of 50 to 60 gram. The suppression of cortical function was confined to the pressure area and was completely reversible in most instances and did not result in significant pathological changes in the affected cortex. It slow waves were present in the electrical activity due to local injury of the cortex, these waves disappeared earlier than did the bursts of rhythmic waves, characteristic of the animal under nembutal anaesthesia. Further experiments showing the use of this method in the differential analysis of various components of the electrocorticogram will be described.

**The effect of thrombin injections on hemostasis**

**MARJORIE B ZICKLER** *Department of Physiology, College of Physicians and Surgeons, Columbia University* In an attempt to study hemostasis in fibrinogenopenic dogs under nembutal, 2000 to 4000 units of Parke-Davis thrombin (125 units/cc) were given through a catheter inserted in the jugular vein (0.18 cc/min) without untoward effects. After injection, blood samples showed less than 10 mg per cent fibrinogen, between 10 and 20% prothrombin activity (Quick method with addition of fibrinogen, calculated from dilution curve), gross hemolysis, normal clot retraction, no fibrinolysis, and a reduction in platelets which however, fell below 150,000/mm<sup>3</sup> in only one of seven experiments. In two experiments the two stage prothrombin method was also used, and showed only a 30% depression of prothrombin. The one stage prothrombin times were increased less by addition of an equal volume of prothrombin-free plasma than by ovalated saline. These data suggest that there is a deficiency of the A<sub>2</sub> factor as well as of prothrombin.

Bleeding times from nicked ear veins were greater than 10 minutes and usually no hemostasis occurred.

curred Glass A-V shunts were not occluded by platelet thrombi although a thin coating frequently formed inside the cannulae. Studies of successive smears from a venous blood sample (Pinniger and Prunty, Brit J Exp Path, 27: 200, 1946) showed smaller groups of platelets and sometimes delayed and diminished metamorphoses. Autopsy failed to disclose microscopic clots or evidence of purpura.

Administration of fibrinogen failed to influence the bleeding time. Whole blood infusion reduced the bleeding time to normal and was followed by occlusion of the shunt.

**Hepato-renal factors in circulatory homeostasis**  
**XVII Relation of renal VEM to vascular compensation in shock** B W ZWEIFACH, S BAEZ (by invitation), R F FURCHGOTT (by invitation) and EPHRAIM SHORR *Dept of Medicine, Cornell University Medical College and The New York Hospital, New York City*. There regularly appears in the blood during the compensatory stage of shock a vaso-ejector principle (VEM) of renal origin which produces in normal test rats hyper-reactive effects on the terminal vascular bed similar to those observed in the same vessels of the shocked animal. Experiments were designed to determine to what extent the compensatory reactions of the peripheral blood vessels during shock are dependent upon participation of the renal VEM mechanism. Anesthetized dogs, subjected to hemorrhage following occlusion of the renal circulation, had no VEM in their blood and developed an accentuated vaso-depressor reaction. VEM was likewise absent from the blood of arenal animals subjected to lethal tourniquet shock and VDM, from the damaged skeletal muscles, appeared in the blood within 10 minutes after tourniquet release. Whereas, in animals with intact kidneys, this VDM is masked in the blood by the preponderance of the oppositely acting renal VEM. The relation of the kidney to the compensatory vascular response to shock was further emphasized by experiments in unanesthetized arenal dogs subjected to a type of hemorrhagic shock from which unanesthetized dogs with intact kidneys are recoverable by transfusion. In the unanesthetized arenal animal, the initial hyper-reactive stage was absent and hypo-reactivity developed with unusual

rapidity. These animals failed to respond to replacement of the blood withdrawn. Microscopic observation of the omental circulation was also used to determine the effect of the exclusion of the kidney on the capacity of the animal to establish adequate compensatory vasoconstrictor changes in the peripheral blood vessels during shock.

**Hepato-renal factors in circulatory homeostasis**  
**XVI Vascular changes in mesentery in renal hypertension in rats** B W ZWEIFACH, S ROSENFELD (by invitation) and EPHRAIM SHORR *(Dept of Medicine, Cornell University Medical College and The New York Hospital, New York City)*. A study was made of the changes in the responses of the terminal arterioles and precapillaries in the mesoappendix during the development of hypertension in the rat following the application of a gauze collodion sac about the kidneys. In control animals the reactivity of the terminal blood vessels to topical epinephrine remains relatively constant, the minimal effective concentration of epinephrine required to produce a slowing of the capillary circulation usually ranging between 1:2 to 1:4 million. Within 2 weeks following the application of a gauze-collodion sac the response to epinephrine becomes enhanced, the threshold concentrations being as low as 1:8 to 1:32 million. Not only is the response to topical epinephrine enhanced but the reaction to intravenously administered VEM is also potentiated. A significant finding during the development of hypertension was the marked increase in the number of capillary vessels in the mesentery which appeared within 2 to 3 weeks after application of the sac to the kidney. Initially all the vessels show an overall rapid circulation. In a later stage a rapid flow persists only through thoroughfare channels, which have a varicose appearance with prominent, thickened, smooth muscle cells. The precapillary sphincters are narrowed so that the capillary flow is sporadic. Little or no vasomotion is seen in the precapillary vessels. The flow in the collecting venules is unusually rapid. These vascular phenomena closely resemble those prevailing during the initial compensatory stage of experimental shock, when VEM predominates in the blood stream.

## THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC

THIRTY-NINTH ANNUAL MEETING

Atlantic City, New Jersey, March 15, 16, 17, 18, 19, 1948

(For possible corrections in any of the following abstracts see the next issue)

**Fractionation studies on the cytoplasmic proteins of human lymphoid tissue** ADOLPH ABRAMS (by invitation) and PHILIP P. COHL, *Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin* A technique for obtaining reproducible electrophoretic patterns of soluble proteins in extracts from human lymphoid tissue (tonsil) has been established. Although complete resolution of all the components of the system is not realized, definite groups of proteins can be identified by a characteristic mobility corresponding to the maximum ordinate of well defined peaks in the pattern. The complex mixture was fractionated with 30, 55, and 100 per cent saturation of  $(\text{NH}_4)_2\text{SO}_4$ , and each fraction was analyzed electrophoretically. Each group of proteins had reproducible solubility properties under the conditions used. A comparison of the solubility properties and mobilities of the tissue proteins with those of serum has allowed a tentative conclusion as to the possible relationship between certain serum proteins and the proteins of lymphoid tissue.

Small amounts of a fast component (mobility about  $12 \times 10^{-5}$  cm<sup>2</sup>/volt/sec.) have been observed consistently. Chemical and spectrophotometric analysis indicate that this fast component is free ribonucleic acid. A fraction of lymphoid tissue prepared by high speed centrifugation, corresponding to the "microsome" fraction was found to have a composition, as to nitrogen and phosphorus, similar to "microsomes" prepared by other workers from different tissues. The ribonucleic acid content of this fraction was of the order of 4 per cent.

**A micromodification of the Folin method for estimating urinary creatinine** ROBERTO REYNAUD ACOSTA (by invitation) and ROBERT E. JOHNSON, *U. S. Army Medical Nutrition Laboratory, 1849 W. Pershing Road, Chicago 9, Illinois* A micromodification of the Folin method for estimating urinary creatinine has been developed for the Coleman Junior Spectrophotometer, Model 6, with #6 310 cuvettes. All reactions are carried out in the cuvette. The only reagent is an aqueous solution containing 0.1% picric acid and 1% sodium hydroxide. Steps in the procedure are: pipette 0.05 ml. urine into cuvette, add 10 ml. of alkaline picrate solution, stand 15 minutes, read, wave length 520. Study was made of the following factors: initial volume of water, concentration of picric acid, concentration of alkali, mixing of picric acid and alkali, time of standing before reading, temperature of reagents, stability of color. Statistical comparison was made

between the modified and the original Folin methods in relation to creatinine standards, urine and urine to which creatinine was added.

**The penicillins produced by *Aspergillus flavus*** M. ADLER (by invitation) and O. WINTERSTEINER, *Division of Organic Chemistry, Squibb Institute for Medical Research, New Brunswick, N. J.* Evidence obtained in an earlier investigation (Fried, Koerber and Wintersteiner, *J. Biol. Chem.*, 103, 341 (1946)) suggested that "flavacidin," the penicillin like antibiotic produced by *Aspergillus flavus* in submerged culture, consisted chiefly of 3-pentenyl penicillin,  $\text{CH}_3\text{CH}=\text{CHCH}_2\text{CH}_2\text{C}_6\text{H}_{11}\text{O}_2\text{N}_2\text{S}$ , a double bond isomer of the 2-pentenyl penicillin (F-type penicillin,  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{C}_6\text{H}_{11}\text{O}_2\text{N}_2\text{S}$ ) elaborated by *Penicillium notatum*. This conclusion was based on the properties of the penilloaldehyde dinitrophenylhydrazone, particularly on the identity of its X-ray diffraction pattern with that of the dinitrophenylhydrazone of synthetic 4-hexenoylaminoacetaldehyde,  $\text{CH}_3\text{CH}=\text{CHCH}_2\text{CH}_2\text{CO}_2\text{NHCH}_2\text{CHO}$ . In contrast, a recently prepared batch of "flavacidin" was found to contain benzylpenicillin (G penicillin) as the main component. The remainder of the active material consisted largely of *n*-amyl penicillin (dihydro F-penicillin), an entity known to be produced also by *Aspergillus giganteus* and by *Penicillium notatum*. In this case the R group present was unequivocally identified by degradation to the acid R-COOH, *n*-caproic acid. It was furthermore ascertained that the dinitrophenylhydrazone of *n*-caproylaminoacetaldehyde (and of the penilloaldehyde obtained from the *n*-amyl penicillin fraction) cannot be differentiated either by melting point or X-ray data from that of 4-hexenoylaminoacetaldehyde. Thus the original conclusion as to the occurrence of a 3-pentenyl penicillin in "flavacidin" has been deprived of most of its basis. The search for an unsaturated (F-type) penicillin in the present material resulted eventually in the isolation of small amounts of an oxidation product, propionaldehyde, which can be derived only from 2-pentenyl penicillin.

**Detoxification of diphtheria toxin by peroxidase** KJELL ACNLER (Introduced by A. M. PAPPENHILMER, JR.) *Chemical Laboratory of the Serafimer Hospital, Stockholm, Sweden, and the Department of Bacteriology, New York University College of Medicine* Previous experiments have shown that both crystalline horse radish peroxidase and purified vegetable peroxidase from leucocytes, in the pres-

ence of 0.001 M hydrogen peroxide, detoxify crude diphtheria toxin produced on a peptone containing medium. It has now been demonstrated that toxin produced on the chemically defined medium of Mueller and Miller is also detoxified by peroxidase. The detoxified material still flocculates with antitoxin and, therefore, has the properties of a toxoid. Highly purified diphtheria toxin was not appreciably detoxified by 0.001 M hydrogen peroxide in the presence of peroxidase. The reaction, therefore, appears to require the presence of some intermediate substance present in crude culture filtrates.

**The sulfur amino acid requirement of the infant.** ANTHONY A. ALBANESE, L. E. HOLT, JR., VIRGINIA I. DAVIS (by invitation), SELMA E. SNYDERMAN (by invitation), MARILYN LEIN (by invitation) and EMILIE M. SNETIK (by invitation). *New York University College of Medicine.* The cystine and methionine requirements of the infant were determined by measuring the nitrogen retention and weight gains on a diet prepared by using a diet deficient in these 2 amino acids as the principal source of nitrogen. This diet was supplemented with varying proportions of L-cystine and L-methionine. Five male infants (4-11 months) were studied. In 3 infants who received no cystine supplement the nitrogen retention and body weight gains were restored to normal levels by an intake of 99 mg of L-methionine per kilo of body weight. In 2 infants who received a 1 per cent L-cystine supplement the nitrogen retention and positive weight change were restored to normal values by an intake of 79 mg of L-methionine per kilo of body weight.

These findings suggest that cystine is capable of stimulating growth only when methionine is present in suboptimal amounts and that about 20% of the methionine needs can be met by cystine. Moreover, it appears from these results that the quantities of sulfur amino acids provided by the commonly employed infant formulae prepared from cow's milk are adequate. On the basis of calculations from the available data on the sulfur amino acid content of human milk, it would appear that the breast milk provides a very meagre margin of safety during the early months of life and that breast milk does not provide an adequate quantity of the sulfur amino acids in the latter part of the first year. The margin of safety is considerably greater in the artificially fed infant.

**Adenine-pentose-pyrophosphate from mung beans.** H. G. ALBAM, M. OGUR (by invitation), H. MENDELSON (by invitation), and A. HIRSCHFELD (by invitation). *Departments of Biology and Chemistry of Brooklyn College, Brooklyn, N. Y.* When a trichloroacetic acid filtrate from fresh mung bean sprouts is neutralized and treated with barium acetate, a precipitate is obtained which, in addition to large amounts of inorganic phosphorus,

contains adenine, pentose, and labile phosphorus. Application of the Needham procedure for the isolation of adenosine triphosphate from this precipitate has yielded unsatisfactory results in our laboratory. If instead, the barium precipitate is suspended in 0.1 M acetate buffer, pH 4.35, and centrifuged, the supernate contains more than 90% of the inorganic phosphorus and about 30% of the adenine. Treatment of the residue with excess sodium sulfate yields a supernate which contains almost all of the remaining adenine. The ratio of adenine:pentose:labile phosphorus:total phosphorus is approximately 1:1:2:3. When this supernate is treated with silver nitrate a precipitate is obtained which, on decomposition with hydrogen sulfide and treatment with barium acetate, yields a barium insoluble fraction with a molar ratio of adenine:pentose:labile phosphorus:total phosphorus of 1:1:2:3. All of the labile phosphorus is split by the potato pyrophosphatase and half is donated to glucose in the yeast hexokinase system at a rate comparable to animal adenosine triphosphate.

**Repletion of protein depleted dogs with whole egg and wheat gluten proteins.** JAMES B. ALLISON, JOHN A. ANDERSON (by invitation), and JOHN I. WHITE (by invitation). *Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey.* Dogs were depleted in protein by feeding a protein-free diet. They were repleted for 30 days by feeding a defatted, dried, whole egg protein or by feeding wheat gluten. The nitrogen balance indexes of the whole egg protein increased above control values in the depleted dog returning to control values upon repletion. Similarly, the index for wheat gluten was greater than control values when the protein was fed to depleted dogs. The excretion of urinary nitrogen on a protein-free diet, (UNo) which reflects the magnitude of the protein stores, decreased upon depletion and returned toward control values with repletion on whole egg protein. Repletion with wheat gluten, however, decreased the UNo even below values obtained during the protein depleted state. Thus, feeding wheat gluten nitrogen did not increase, rather decreased, those protein stores which are involved in the formation of minimum catabolic nitrogen. These results may be interpreted to mean that body protein stores may be utilized to supplement the wheat gluten, an interpretation which is supported by data obtained in normal dogs. Some tissue stores were repleted with the wheat gluten, however. The body nitrogen gain and the plasma protein nitrogen gain with wheat gluten was equal to approximately half that obtained with whole egg which increased body nitrogen and plasma protein nitrogen almost to control values.

**The rate of disappearance of human albumin in normal and protein depleted dogs.** CARL ALPER

(by invitation) SHIRLEY DeBIASE (by invitation) and BACON F CHOW *Division of Protein Chemistry, The Squibb Institute for Medical Research, New Brunswick, New Jersey* The study of the rate of disappearance of a foreign protein from the circulation, although of fundamental importance in protein metabolism, has been neglected generally because suitable quantitative techniques for the estimation of the foreign protein were not available. Recently, an immunological method was described for the quantitative determination of human albumin. This method is satisfactory for the estimation of human albumin in the presence of other albumins. Normal dogs and dogs depleted by plasmapheresis were injected with approximately 250 mgm of normal human serum albumin per kgm body weight. Samples of blood were removed at fixed time intervals for 96 hours and the amount of serum albumin in the circulation was determined by the immunological method and by the determination of total plasma volume. The serum albumin is tolerated very well by the normal and depleted dogs. There is no significant change in plasma volume after the injection of serum albumin. The disappearance of serum albumin is rapid in the first 24 hours and then the rate gradually becomes asymptotic. Approximately one-third of the infused albumin disappears in 24 hours, and an additional one third disappears in the next 72 hours.

**Aminoaciduria in progressive muscular dystrophy** STANLEY R AMES and HUGH A RISLEY (introduced by P L HARRIS) *Laboratories of Distillation Products, Inc., Rochester, New York* Wasting of muscle is characteristic of progressive muscular dystrophy but no significant changes in total nitrogen excretion are observed. It seemed probable that there might be an increased urinary excretion of minor nitrogenous products in addition to creatine, such as free amino acids. The use of partition chromatography to determine the urinary distribution and excretion of amino acids in humans was developed by Dent (Lancet, II, 637 (1946)). He reported low concentrations of two and occasionally three or four amino acids in urine of normal humans. A total of 30 urine samples were obtained from patients who had been diagnosed as suffering from progressive muscular dystrophy. The urines were chromatographed on filter paper employing the "strip" technique with the following solvents saturated with water: butanol, phenol, and lutidine-collidine (50-50). The results of the "strip" chromatograms indicated that practically all urines from patients not receiving therapy were markedly increased in amino acid excretion as compared with normals. Two dimensional partition chromatography was also employed using phenol and lutidine-collidine (50-50) as solvents. Compared with a normal picture of from 2 to 4 spots on

the chromatogram, the majority of urines from patients not receiving therapy showed 8 to 11 or more spots. As many as 15 separate spots have been observed. On the basis of these urine specimens, it can be concluded that the condition of human progressive muscular dystrophy is characterized by a generalized aminoaciduria.

The estimation of functional hepatic mass in normal and phosphorus poisoned dogs. CARL E ANDERSON (by invitation), ROBERT G GALE (by invitation) and C S ROBINSON *Departments of Biochemistry and Medicine, Vanderbilt University, Nashville, Tennessee* Mason has reported (Federation Meeting, May 1947) a constant injection technique for estimating the functional hepatic excretory mass for dogs based on the principle that the maximum rate of hepatic excretion of bromsulphalein (Lm) is proportional to the functional hepatic mass. His values for Lm ranged from 7.5 to 10.3 mg/min/sq meter surface area, and averaged 0.15 mg/kg body wt/min. We have obtained values from 5 normal dogs (15 experiments) of average weight (10-13 kgs) which confirm Mason's constants.

| Dog | Lm         |                   |
|-----|------------|-------------------|
|     | Mgs/kg/min | Mgs/min/sq m S.A. |
| 1   | 0.43       | 9.5               |
| 2   | 0.47       | 9.1               |
| 3   | 0.46       | 10.8              |
| 4   | 0.42       | 8.3               |
| 5   | 0.42       | 8.9               |

Two dogs poisoned by the daily subcutaneous injection of phosphorus (0.4 mg/kg/P<sub>4</sub>) showed reduction in Lm. Dog 2 died when a value of 5.8 mg/min/sq m S.A. (0.28 mg/kg/min) was obtained. Gross examination of the liver revealed two areas of hemorrhagic degeneration in the parenchyma. Severe liver necrosis was observed histologically. Dog 5 was sacrificed when the value for Lm reached 6.7 mg/min/sq m S.A. (0.31 mg/kg/min). Histological examination of the liver revealed only a slight cloudy swelling. The prothrombin time remained essentially normal. Present observations indicate that values representing a functional change in the liver without apparent histological change may be obtained.

**On the fate of labeled pyruvic acid in the intact animal** H S ANKER (introduced by E A EVANS, JR.) *Department of Biochemistry, University of Chicago* Pyruvic acid labeled with C<sup>14</sup> at carbon atom 2 was synthesized from C<sup>14</sup> carbon dioxide via acetic acid, pyruvitrile and pyruvic acid amide. Pyruvic acid was injected intraperitoneally over a period of 3 days into a rat which was kept on a high protein diet. p-Amino benzoic acid was fed simultaneously. The following compounds were isolated



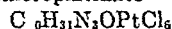
and their  $C^{14}$  content determined. They are given in the order of decreasing radioactivity: Urea, acetyl p amino benzoic acid, liver protein, glycogen, saturated liver fatty acids, liver cholesterol, saturated carcass fatty acids, carcass cholesterol and hemin. From the radioactivity of the isolated compounds it can be concluded that the carbon atoms of pyruvic acid are utilized in the synthesis of all the compounds mentioned. The bulk of the recovered  $C^{14}$  was found in the fatty acids.

**Partial hydrolysis products of human hair**  
JAMES C. ANDREWS, *Department of Biological Chemistry and Nutrition, School of Medicine, University of North Carolina, Chapel Hill, N. C.*  
Slow hydrolysis of human hair by means of 3 M  $H_2SO_4$  at  $38^\circ C$  produces a solution of proteose-peptone corresponding in amount to about half the original weight of the hair. The protein in the solution has a free amino to total nitrogen ratio of about 39%, a cystine sulfur content of 2.90% and an optical activity of  $-50$ . Variations in the time of hydrolysis from 4 months to over 4 years yield a product in which the above figures remain very constant. The total non sulfate sulfur of these solutions is about twice the amount of cystine sulfur. The evidence indicates that the non sulfate, non cystine sulfur is partly, if not all, in the form of cysteic acid. This latter has evidently resulted from atmospheric oxidation of cystine produced in amounts too large to find place in the structure of the proteose and the peptone. The insoluble residue from such hydrolyses still retains the gross and microscopic appearance of the original hair. However, it is highly brittle and easily crumbles to a powder. It contains about 12% nitrogen and 5.2% sulfur but produces no free cystine on complete hydrolysis.

**Fractionation of urinary steroids by use of the Craig counter current distribution machine**  
REGINALD M. ARCHIBALD and E. STROH (by invitation), *Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland*. Carbon tetrachloride extracts of hydrolyzed, human urines from normal individuals and from those with disturbed production or metabolism of hormones of the suprarenal glands or gonads have been fractionated in the Craig machine using equal volumes of cyclohexane and 1.38% (by volume) solution of alcohol in water. Analysis of fractions by use of a) spectrophotometric determination of the absorption of ultra violet light, b) photofluorometric measurement of the fluorescence produced on heating fractions with 90%  $H_2SO_4$ , c) Zimmermann's reaction with alkaline m-dinitrobenzene showed the presence of 8 or more components. Attempts are being made to effect further fractionation and identification of the components. The fluorescence resulting from heating fractions with 90%  $H_2SO_4$  paralleled the ab-

sorption of light of  $\lambda = 410 m\mu$  by these heated fractions.

**Reaction of plasmochin with formaldehyde**  
R. M. ARCHIBALD and JAMES R. WEISIGER (by invitation), *Hospital of The Rockefeller Institute for Medical Research, New York*. An understanding of the degradation of plasmochin *in vivo* is basic to an understanding of its pharmacological action. It reacts readily in aqueous formaldehyde solution binding irreversibly one equivalent of acid and one molecule of formaldehyde. The structure of the strongly fluorescent quaternary salt which can not couple with diazo reagents and which is 20 times more toxic than plasmochin was confirmed by analysis of the chloroplatinate



Calc. C 32.59, H 4.23, N 5.69

Found. C 32.28, H 4.16, N 5.67

This compound decomposes irreversibly in alkaline solution to give at least two new compounds, neither of which is soluble in non-polar solvents, suggesting the persistence of the quaternary structure. These properties have prevented the recovery of any of the products from admixture with biological material. A comparable *in vivo* reaction product would not be detected by the ordinary methods of isolation and analysis, in accord with the observed rapid disappearance of plasmochin from the blood. The toxicity of the drug to parasite or to host may result from reaction with a naturally occurring aldehyde or ketone.

**Tissue incorporation and excretion of radioactive carbon administered as carbonate**  
W. D. ARMSTRONG, JACK SCHUBERT (by invitation), and ARTHUR LINDENBAUM (by invitation), *Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minnesota*. The excretion of  $C^{14}$  in expired air, urine and feces by mature rats has been determined over periods of 6-40 days after intraperitoneal injection of  $Na_2C^{14}O_3$  and after intraperitoneal implantation of  $CaC^{14}O_3$  as a powder or as pellets. The absorption of  $C^{14}$  from pellets of  $CaC^{14}O_3$  is slow and allows a greater incorporation of the isotope in organic compounds and in the bone salt. In 40 days all but  $0.38 \pm 0.053\%$  of  $C^{14}$  administered as powdered  $CaC^{14}O_3$  is excreted. These data and the results indicating the fraction of the total dose of  $C^{14}$  found in the skeleton, enamel, dentine, bone inorganic carbon, bone protein, liver and muscle glycogen, glycerol, fatty acids, muscle protein, hemin and in other tissues and compounds will be presented.

**The action of ethanolamine, monomethyl- and dimethyl-ethanolamine on lipid phosphorylation**  
CAMILLO ARTOM and W. E. CORNATZER (by invitation), *Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina*. Rats maintained for 7 days on a low fat, low protein diet received by

stomach tube a single dose (0.2 millimoles in 1 cc of water) of ethanolamine, monomethyl-, dimethyl-ethanolamine or choline. Controls received 1 cc of water. The rats were then injected with  $P^{32}$  (as  $Na_2HPO_4$ ). After 6 hours the lipides were extracted from the liver and small intestine and their radioactivity and phosphorus content were determined. All substances tested stimulated lipide phosphorylation in both tissues. After giving ethanolamine, monomethyl-, dimethyl-ethanolamine, and choline, the average increases in the specific activity above the controls were 13, 65, 56 and 21 per cent in the liver, and 13, 20, 36 and 16 per cent in the intestine, respectively.

In a few experiments the separation of the liver phospholipides into choline-containing (C c) and non choline-containing (N c c) was attempted (see Taurog et al., *J Biol Chem*, 155, 19 (1941)). In almost all experiments increases in the radioactivity of both fractions were observed. However, the increase was proportionately greater in the N c c fraction after giving ethanolamine. It was approximately the same in both portions after feeding monomethyl-ethanolamine. On the other hand, it was definitely greater in the C c fraction when dimethyl-ethanolamine and especially when choline had been administered.

**On the absorption of phospholipides** CAMILLO ARROY and MARJORIE A. SWANSON (by invitation) *Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina*. Labelled phospholipides (obtained from the livers of rats injected with radioactive phosphate) were fed by stomach tube to fasting rats. After 3 or 6 hours the radioactivity and phosphorus content were determined in the inorganic and lipide fractions of plasma and liver. The results were compared with those obtained on rats receiving by stomach tube non-labelled phospholipides and  $P^{32}$  as sodium phosphite. In these controls, at both time intervals, the specific activities were in the following order: liver inorganic P > plasma inorganic P > liver lipide P  $\geq$  plasma lipide P. These findings are consistent with the assumption that the phospholipides synthesized in the liver from the absorbed inorganic phosphate were the main or only source of the labelled lipides in plasma. On the other hand, in all experiments in which radioactive phospholipides were fed, the gradient of the specific activities was the following: plasma lipide P  $\geq$  plasma inorganic P  $\geq$  liver inorganic P > liver lipide P. The presence after 3 hours of large amounts of isotopic phosphorus in the inorganic fraction of the liver and especially in that of the plasma indicates that the phosphate was rapidly split from the lipide in the intestinal tract and absorbed. However, the high specific activity of the phospholipides in plasma is definite evidence that they cannot derive solely from the

liver lipides, but that a detectable amount of the ingested phospholipides has also been absorbed as the intact molecule.

**Folic acid requirement of the rat and some characteristic lesions observed in the deficient animals** CONRADO F. ASHBY *Department of Chemistry and Nutrition, School of Tropical Medicine, San Juan, Puerto Rico*. White rats (Wistar Institute strain), fed since their 21st day a highly purified diet, high in carbohydrate and containing 2% sucinylsulfathiazole, besides all the known nutritional requirements except folic acid, showed cessation of growth, leukopenia, and the characteristic skin lesions of folic acid deficiency in an average of 1 to 5 weeks. These animals were supplemented at this time with different levels of folic acid administered 6 times per week for a period of 3 weeks. One group was left as negative controls. Gain in weight, leukocyte response, and per cent survival were recorded.

According to these results, the minimum amount of folic acid required by these strains of rats for maintenance is in the neighborhood of 0.5  $\mu$ g per day six times per week. However, this amount of folic acid does not improve the white cell count during the supplementation period. To make appreciable gains in weight and to recover the normal white cell count in the 3 week period these rats seem to require somewhere between 1-8  $\mu$ g per day. All negative controls, as well as 33% of animals receiving 0.5  $\mu$ g of folic acid, were found at autopsy to have developed infarcts in the spleen. None of the animals receiving 1  $\mu$ g, or more, of folic acid per day developed this lesion. The immediate cause of these infarcts, according to Dr Enrique Koppisch, of our Department of Pathology, seems to be a thrombus formation in the splenic vein.

**Distribution pattern of carbonic anhydrase in the fetal central nervous system** WINFRED ASHBY (introduced by Dr JOSEPH H. ROE) *Blackburn Laboratory, St. Elizabeths Hospital, Washington*. The quantitative distribution of carbonic anhydrase in the central nervous system has been studied in 7 prematurely born human infants and one full term, and in 5 fetuses, obtained from cattle slaughtered for beef, ranging in age from 3 to 7½ months. As distinguished from the adult pattern of distribution, carbonic anhydrase was not found in the cerebrum of the fetus, although it occurred in measurable and in progressively increasing amounts in phylogenetically older parts of the central nervous system. In the human fetus the comparison between the content of the medulla and the pons, which in the adult gave an excess in the pons, showed an excess in the medulla in the fetus. In two instances in which the cord was obtained a markedly greater content was found in the cord than in any other part studied as contrasted with the adult in which higher centers

greatly exceeded the cord in carbonic anhydrase content. That the lack of carbonic anhydrase content in the cerebrum of the premature human infants, which did not survive, was not abnormal, would seem to be supported by the negative findings in the cerebrum of the cattle fetus examined up to 7½ months of fetal life. In a calf a few days after birth a cerebral content of 7.8 units was found as compared with 13.5 units in the hemispheres of the mature animal.

**Effect of carbon dioxide concentration on brain lactate.** JAMES A. BAIN (by invitation) and J. RAYMOND KLEIN, *Departments of Pharmacology, Psychiatry, and Biological Chemistry, University of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago*. In cats, it was found that the following experimental conditions: 1) breathing mixtures containing 5-30% carbon dioxide and 95-70% oxygen, 2) breathing carbon dioxide mixtures during intravenous administration of sufficient sodium bicarbonate to maintain the plasma pH at a control level, and 3) breathing oxygen during administration of bicarbonate result in concentrations of brain lactate that are lower than the level found in cats breathing room air. Compared to the brain lactate level found in air, the experimental conditions produced decreases in level of about 60, 30, and 10% respectively. The results suggest that the effect of carbon dioxide is not due to change in plasma hydrogen ion concentration alone, but depends also upon the concentration of total carbon dioxide, i.e. carbon dioxide plus carbonic acid. It is not clear as yet whether known effects of increased carbon dioxide concentration, i.e. increase in cerebral blood flow and consequently in oxygen tension, account completely for the changes in brain lactate or whether other factors are involved.

**Circulatory disturbances in hepatic and renal cortical necrosis.** JAMES H. BAXTER (introduced by DONALD D. VAN SLICK), *Hospital of The Rockefeller Institute for Medical Research, New York*. On the basis of previous observations in rats, it was suggested that pyridine liver necrosis might be due to disturbance of circulation through the lobules, resulting in anoxia of the centro-lobular cells, rather than to direct necrotizing action (Am. J. Path., in press), and that renal cortical necrosis of choline deficiency might likewise result from circulatory disturbances (J. Nutrition, 31: 333, 1947). Further observations indicated below, are at least compatible with these suggestions. Necrosis occurred earliest in those portions of the functional units furthest removed from the source of blood supply, while cells about portal triads in the liver, and the glomeruli and structures in the "lesser" renal circulation escaped. It was demonstrated by India ink injections that little circulating blood entered the involved areas. Dead cells

were slowly resorbed. Necrotic regions became engorged with deoxygenated blood, involved organs enlarged, dark and tense. In terminal stages of irreversible damage, marked obstruction to perfusion of fluid through the injured organ as a whole occurred. Shock often preceded death due to liver necrosis. Observations at various stages of injury have not definitely established the immediate cause of the circulatory disturbance, or that the disturbance precedes necrosis. Decapsulation of the organs has not produced conclusive protection. Others (Himsworth, Hartroft) believe compression of vessels by swollen but viable parenchymal cells initiates necrosis under similar circumstances. The circulatory disturbances, it appears, if not actually initiating necrosis, may at least be responsible for extension of the necrosis and further damage to the organism.

**Correlation of the unitarian or trophoblastic thesis with the biological test of malignancy.** HOWARD H. BEARD, *Department of Biochemistry, The Chicago Medical School, Chicago, Ill.* The correct etiology of malignancy and nature's remedy for it were first announced in 1902 by John Beard of Edinburgh. His trophoblastic thesis states that the wandering germ cells of early life can later be activated to divide and produce trophoblast cells which, outside the canalization of pregnancy, are the malignant cells. Since the pregnancy trophoblast begins to disappear about the time the fetal pancreas develops, he also suggested the use of pancreatic extracts for the treatment of human malignancy. It is most unfortunate for the human race that these extremely important discoveries had to lie dormant until Gurchot, Krebs and Krebs recently confirmed and greatly extended the theories and treatment of malignancy advocated by Beard. The Asheim-Zondek test is positive in most genital cancers and in some nongenital ones. Our biological test is a micro AZ test and we have obtained positive results in 130 of 134 malignant urines tested in the last 2 years. Since the malignant (or pregnancy) trophoblast gives off syncytial steroids and chorionic gonadotrophin, these malignant hormones are extracted from the urine by alcohol ether and, after removal of the solvents, these extracts cause hypertrophy of the spleen and/or gonads 2½ days after injection into immature rats. It is, therefore, concluded that the results of these biological tests constitute the strongest experimental evidence for the validity of the unitarian or trophoblastic thesis of malignancy.

**Effect of pH on the aerobic metabolism of rabbit bone marrow.** ROBERT M. BIRD and JOHN D. EVANS (introduced by WILLIAM H. SUMMERSON), *Department of Physiology, Cornell University Medical College, New York, N. Y.* The aerobic metabolism of the bone marrow cells of New Zealand White rabbits had been studied *in vitro* in

bicarbonate-containing media within the pH range 7.6 to 6.3. Between pH 7.4 and 7.1 oxygen consumption averaged 3.3 microliters per mgm of cell protein per hour and showed little alteration with change in pH. From pH 6.8 to 6.3 oxygen consumption averaged 2.7 microliters per mgm per hour and again was little influenced by pH changes within this range. At these two rates of oxygen consumption the average R/Q's were 0.97 and 0.95 respectively. Aerobic acid production was more affected by change in pH, being depressed in a substantially linear fashion with increasing acidity. At pH 7.4 it averaged 3.9 microliters per mgm per hour, at pH 6.3, 1.4 microliters per mgm per hour. Lactic acid formation accounted for approximately 90% of all acid produced aerobically throughout the pH range studied. Glucose utilization, in the majority of experiments, was sufficient to account for both lactic acid formation and total oxygen consumption. These findings are correlated with prior studies on bone marrow metabolism.

**Effects of pH and bicarbonate on brain tissue respiration and anaerobic glycolysis.** MARION K. BIRMINGHAM (by invitation) and K. A. C. ELLIOTT, *Montreal Neurological Institute, McGill University*. Previously it has been shown (Fed. Proc. 6, 249) that there is a broad optimum pH for brain tissue respiration at about 7.1, and that apparent high optima found with slices are due to the fact that the pH of the slice is not the same as that of the medium. The anaerobic glycolysis of isotonic brain tissue suspensions has now been found to show a rather sharp optimum at about 6.8. With careful equalization of pH it was found that, compared to unbuffered Ringer solution, bicarbonate-buffered Ringer causes a slight inhibition of respiration, about 7% with 0.02 M bicarbonate 5% CO<sub>2</sub>, about 16% with 0.04 M bicarbonate 10% CO<sub>2</sub>. Small amounts of bicarbonate added to phosphate buffered medium in the presence of alkali papers appear to increase the oxygen uptake rate but this can be accounted for by an artefact arising from changing CO<sub>2</sub> tension. The rates of glycolysis in phosphate and bicarbonate buffered media of equal pH are about the same, and lactic acid accounts approximately for all of the acid produced in either medium. A large difference between bicarbonate and phosphate buffered media reported previously (Elliott and Henry, J. Biol. Chem. 163, 361) was due to an error. Increasing bicarbonate concentration with constant CO<sub>2</sub> tension depresses the rate of glycolysis, but this can be accounted for by the increase in pH above the optimum.

**A microanalytical method for acetic and other volatile acids.** SIMON BLACK (introduced by E. S. GUZMAN BARRON), *Chemical Division, Department of Medicine, The University of Chicago*. A simple microdiffusion and microtitration procedure for volatile acids has been substituted for the usual

steam distillation and macro- or semi microtitration. Quantities of acetic acid between 10 and 100 micrograms, or equivalent amounts of other volatile acids, can be determined with an average error of less than 2 micrograms. Large numbers of analyses can be performed simultaneously. To determine acetic acid in the absence of interfering substances 1 ml. of a protein free filtrate of the sample is made slightly alkaline and evaporated to dryness (105°) on the bottom of a 30 ml. bottle. A small glass cup (12 mm O.D., 5 mm deep) containing 25 c.mm. of 0.1 M NaHCO<sub>3</sub>-15% NaI is centrally placed on the floor of the bottle. One half ml. of 15 N H<sub>2</sub>SO<sub>4</sub> saturated with Ag<sub>2</sub>SO<sub>4</sub> is pipetted onto the floor of the bottle outside the cup. A cap with a tinfoil-covered rubber gasket is screwed on tightly and the bottle placed in an oven (105°) over night. Thirty five c.mm. of 0.1 M K<sub>2</sub>HPO<sub>4</sub> 0.03% Duponol PC is added to the center cup (removed from bottle) to eliminate CO<sub>2</sub>, then 3 drops of saturated phenolphthalein solution (in 1% LiOH) and a short (5 mm.) glass covered iron wire (magnetic stirrer). The solution is titrated over a revolving magnet with 0.1 N NaOH from a microburette. The latter is constructed from a tuberculin syringe and a micrometer caliper. Acetic acid is calculated from the difference between sample and blank titrations.

**Synthesis of fatty acids in rat liver slices.** ROYALD BLOCH and W. KRAMER (by invitation), *Department of Biochemistry and Institute of Radiobiology and Biophysics, University of Chicago*. Incubation of rat liver slices in the presence of labeled acetic acid leads to extensive incorporation of isotope into cholesterol, but little isotope enters into fatty acids under these conditions (J. Biol. Chem. 162, 441 (1945)). Present experiments show that fatty acid synthesis in surviving rat liver is greatly stimulated by addition of pyruvate to the medium. Fatty acids formed from CH<sub>3</sub>C<sup>14</sup>OO<sup>-</sup>Na contain 4-8 times more C<sup>14</sup> in the presence than in the absence of pyruvate. The same stimulatory effect is shown by oxaloacetate but not by glucose, fumarate, malate or succinate. In bicarbonate buffer more than twice as much isotope is incorporated into fatty acids than in phosphate buffer. In the absence of calcium ions fatty acid synthesis is reduced to insignificant levels. Under conditions which are favorable for fatty acid synthesis, the rate of cholesterol synthesis is diminished. Utilization of acetate for cholesterol formation is reduced to less than half of the control value by addition of pyruvate or oxaloacetate.

**The inhibitory effects of  $\alpha$ -amino acids on phosphatase activity.** OSCAR BODANSKY and NORMA STRACHMAN (by invitation), *Department of Pharmacology, Cornell University Medical College, New York City*. It was previously shown that glycine, in concentrations higher than the 0.006

is necessary in conjunction with other conditions for optimal activity, inhibited bone and intestinal phosphatases, the former entirely in a non-competitive manner and the latter largely so (Bodansky, O, J Biol Chem, 165, 605 (1946)) The inhibition was dependent on the presence of the free carboxyl and amino groups of glycine In the present report, the inhibitory effects of varying concentrations of other  $\alpha$  amino acids on phosphatase activity is studied at 25°C, at optimal alkaline pH, at optimal concentrations of  $Mg^{++}$  and of glycine (0.006 M) and at near maximal or maximal concentrations of the substrate, Na  $\beta$  glycerophosphate The reaction velocities of rat intestinal, human intestinal, rat bone and cat bone phosphatases were reduced to 50% of their uninhibited values by, on the average, 0.11 M dl-alanine or 0.05 M glycine Although there was some variability, there appeared to be no significant difference, for either amino acid, between the effects on the bone and intestinal phosphatases of these species Other  $\alpha$  amino acids not only exerted inhibitory effects of a different order of magnitude, but also showed differences with respect to rat bone and intestinal phosphatases The following concentrations of amino acids produced 50% inhibition in rat intestinal and bone phosphatases, respectively 0.03 M and 0.09 M l glutamic acid, 0.21 M and 0.01 M l lysine, 0.006 M and 0.003 M l histidine

The role of bicarbonate in glutamic acid metabolism ERNEST BOREK and HEINRICH WAELSCH Departments of Biochemistry New York State Psychiatric Institute and Columbia University Bicarbonate has been shown to interfere with glutamic acid metabolism in *Lactobacillus Arabinosus*, apparently by blocking the amidation to glutamine (Waelseh, Prescott and Borek, J Biol Chem 171) The amount of bicarbonate needed for complete growth inhibition varies with the pH of the medium and with the glutamic acid concentration At pH 6.3 and glutamic acid concentrations of 40, 80 and 120 gamma per ml, 1.2, 2.4 and 2.8 mgm of bicarbonate per ml inhibit growth completely (molar ratios 5:1, 5:1 and 4:1) At this pH the addition of 0.4 gamma of glutamine per ml to the medium containing 120 gamma of glutamic acid overcomes the growth inhibition caused by 2.8 mgm of bicarbonate Bicarbonate also inhibits if ketoglutaric acid is substituted for glutamic acid and this inhibition is also overcome by small amounts of glutamine 2 mg of cysteine per ml overcome completely the inhibition induced by 2.8 mgm of bicarbonate per ml The possible role of carbonic acid as the regulator of the utilization of glutamic acid either for amidation or for transamination is discussed Observations on this mechanism in mammalian tissues are presented

Protein and peptide turnover with respect to

lysine in guinea pig liver homogenate HENRY BORSOOK, CLARA L DEASY (by invitation), JACOB W DUBNOFF, C T O FONG (by invitation), WILLIAM D FRASER (by invitation), A J HAAGEN SMIT (by invitation), GEOFFREY KEIGHLEY (by invitation), PETER H LOWY (by invitation) William G Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena 4, California Protein and peptide turnover in guinea pig liver homogenate was studied with the aid of lysine synthesized with  $C^{14}$  in the  $\epsilon$ -position The homogenate was incubated with an amino acid mixture (final concentration 1%) of the composition of casein in which all the added lysine was labelled, and  $\alpha$ -ketoglutarate (final concentration 0.01 M) The pH varied from 7.5 to 9.0 in different experiments After 6 hours at 38° under oxygen the proteins were removed and a peptide fraction containing lysine was isolated as the phosphotungstate Its insolubility in acetone distinguishes it from the phosphotungstate of lysine Isolation and purification procedures excluded the adsorption of unbound radioactive lysine The lysine isolated from the protein after hydrolysis contained a small amount of radioactivity, indicating less than 0.1% turnover The peptide fraction contained 5 per cent of the added lysine The turnover in this fraction was therefore very high Chromatography of the peptide fraction on filter paper gave, before hydrolysis, one spot with ninhydrin, after hydrolysis, 6 or more spots The free  $\alpha$  amino nitrogen before and after acid hydrolysis indicated that there might be approximately 8 peptide bonds The quantity of the peptide fraction available at present is too small for a complete amino acid analysis, indications are that it contains lysine, arginine, histidine, and mono amino acids Tryptophane and tyrosine are absent A similar peptide fraction is normally present in guinea pig liver

Inactivation of trypsin by acetylation and iodination DONALD E BOWMAN Department of Biochemistry and Pharmacology, Indiana University, School of Medicine, Indianapolis, Ind Acetylation of trypsin by means of ketene decreases its proteolytic activity and the hypotensive effect of its intravenous administration With increasing acetylation the decline in proteolytic activity is no less rapid than the decrease in hypotensive effect This is in contrast with the more rapid loss of hypotensive activity which results from iodination as reported earlier Loss of activity upon acetylation does not appear to be due to the blocking of amino groups The tyrosine color value decreases to about half of that of untreated trypsin when the hypotensive and proteolytic activities have decreased to one tenth of their original values Considerable recovery of proteolytic activity as the result of mild alkaline hydrolysis of acetyl trypsin

indicates that loss of this activity is at least in part associated with the linkage of acetyl groups to oxygen such as that of the phenolic hydroxyl groups. Iodination of trypsin with constant amounts of iodine less than that required to satisfy all groups has less influence on the hypotensive effect with a decrease in pH which slows the rate of iodination of groups such as histidine more than that of tyrosine. Groups which iodinate rapidly at decreased pH levels such as cysteine and tryptophane do not appear to be involved in inactivation. Failure of the two types of activity of the enzyme to remain parallel is in accord with the ability of navy bean trypsin inhibitor to inhibit proteolysis without preventing a fall in blood pressure following intravenous injection.

**The glucose content of the rat carcass.** MURICE BRUGER, SAMUEL MENBIR (by invitation) and ESTELLE GOLDMAN (by invitation) *From the Medical Research Laboratory, Department of Medicine, New York Post Graduate Medical School and Hospital.* The total glucose content of the carcass was determined in 98 young rats (Wistar strain) as control observations for studies on alloxan diabetes. Detailed precautions were taken to avoid glycolysis and the whole animal macerated in a special crusher (packed in dry ice). Somogyi filtrates were prepared and glucose determined by the Shaffer-Hartmann method. Subgroups were studied in which the pre-analytic preparations differed as follows: (a) Purina Chow Checkers or low carbohydrate diet; (b) catharsis; (c) evisceration; (d) 24-hour, overnight or no fasting. Carcass glucose decreased as the starvation period was prolonged, fasting for 24 hours gave a high mortality rate in young rats. Purina chow was too high in carbohydrate content and gave irregular results because of variable amounts of food remaining in the gastro-intestinal tract even after catharsis (castor oil). Eviscerated animals (stomach and intestines removed) gave the highest glucose values averaging  $99.0 \pm 12.2$  mgm per 100 grams of rat. The most consistent results in the whole animal were obtained in a group of 34 rats ranging in weight from 39.8 to 66.8 grams on a low carbohydrate diet (J Nutrition 10:507, 1935, diet 10) for one week and fasted overnight. In this group, the average glucose content of the carcass was  $53.0 \pm 19.2$  mgm per 100 grams of rat.

**Reversible oxygenation, irreversible oxidation, and decarboxylation of cobalt-amino acid complexes with oxygen gas.** DEAN BURR, and (by invitation) SILVIO FIALA, JOHN HEARON, MARIE HESSELBACH, HILTON LEVY, and ARTHUR L. SCHADE. *National Cancer Institute, National Institute of Health, Bethesda, Maryland, and Overly Biochemical Research Foundation, New York.* Various cobaltous amino acid complexes, in addition to cobaltodihistidine and certain cobaltous

histidine derivatives reported on previously (J Biol Chem 165:723 (1946), Fed Proc 6:242 (1947)), react reversibly with oxygen gas under physiological conditions. Reversibly oxygenated, yellow-brown cobaltous compounds were readily formed with lysine, arginine, tryptophane, proline, ornithine, serine, asparagine, glutamine, glutamate, glycine, and glycyl glycine at concentrations of the order of 0.01 to 0.1 M. However, the oxygenated compounds were transformed spontaneously into pink, irreversible oxidation forms, many times more rapidly than was oxybis(cobaltodihistidine). In general, the stability of the oxygenated forms decreased with increasing temperature and pH. Irreversible  $O_2$  consumption, but no intermediate reversible oxygenation, was observed with cysteine, methionine, alpha alanine, and putrescine, and no oxygen consumption at all with beta alanine.

The irreversible reaction is complex, and may be shown with histidine to involve several recognizable phases, including 1) an initial irreversible rearrangement of the reversible form without additional  $O_2$  uptake, 2) eventual absorption of a second molecule of  $O_2$  by oxy-bis(cobaltodihistidine), and 3) especially with excess amino acid, production of  $CO_2$  and  $OH^-$ , in ratios of  $CO/O$  and  $OH^-/O$ , approaching maxima of 0.5 and 1 respectively, indicating complex organic oxidation and decarboxylation. With excess cobalt it was possible to obtain complete conversion to a pink irreversible form without notable production of  $CO_2$  or  $OH^-$ , indicating non-oxidation of the cobaltous to cobaltic state here, a result further confirmed polarographically, where no change in height or potential of the cobaltous cobalt wave was observed upon conversion of cobaltodihistidine to oxy-bis(cobaltodihistidine) and then to the pink irreversibly oxidized complex.

**A study of the microbiological assay of valine with estimation of precision of the method.** MURIEL M. BURR and W. A. CRANDALL (introduced by L. I. PUGSLEY). *Food and Drugs Division, Department National Health and Welfare, Ottawa, Canada.* A synthetic medium of pure amino acids and vitamins is described for the assay of valine, employing *Lactobacillus rhabdus* as the test organism. Comparisons are made with other proposed methods using this organism. Comparable results are obtained in the assay of various protein hydrolysates, pharmaceuticals and proteins and recovery experiments were satisfactory. An estimation of the precision of the method is carried out according to Crandall and Burr "Precision of microbiological assays for riboflavin, niacin and pantothenic acid" (in press).

**The metabolism of peptides of asparagine.** C. E. CARTER (introduced by J. P. GREENSTEIN). *Clinton National Laboratory, Oak Ridge, Tennessee.*

see Enzymes capable of hydrolyzing the peptide bond of glycyl l-asparagine without attacking the amide group have a high activity and wide spread distribution in mammalian tissues. The optimum effect exhibited by this system is at pH 8. Liver and kidney extracts are the most potent sources of the enzyme, and in dilute solution effect the complete hydrolysis of the peptide in  $2.8 \times 10^{-4}$  M substrate in 20 minutes. Dialysis does not inactivate the enzyme system and there is no specific metal activation, or anion effect such as has been described in the desamidation of glutamine and asparagine. Leucyl Asparagine is metabolized at the same rate as glycyl asparagine. Chloroacetyl asparagine,  $\alpha$  bromisocaprolyl asparagine, are metabolized by aqueous tissues extracts but at a much slower rate. Hydrolysis of the peptide bond has been followed manometrically by the chloramine T reaction and by the ninhydrin reaction. A purification of the enzyme system has been effected from rat liver by ammonium sulfate fractionation of aqueous extracts.

Influence of dietary and physical factors on the urinary excretion of thiamine and pyrimin. W. O. CASTER (by invitation), OLAF MICKELSEN and ANCEL KEYS. *Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis*. One year 4, 4, and 2 normal young men were maintained on constant thiamine intakes of 0.6, 1.0, and 1.8 mgm per day respectively for 5.5 months. In another year 6 and 6 men were on 1.0 and 2.0 mgm of thiamine per day respectively for 8 months. The 24 hour urinary thiamine and pyrimin excretions were followed throughout each period. On separate occasions each lasting 5 days while the thiamine and caloric intakes were kept constant, a diet providing 200 grams of protein, and one supplying 80% of the calories from carbohydrate were fed. In both of these periods, the urinary thiamine and pyrimin excretions showed no consistent and significant changes over that of the preceding control period. A diet providing 75% of the calories from fat was fed for 5 days one year and for 15 days the second year. In both cases there was an indication of a significant increase in the thiamine and pyrimin excretions. This significance, however, was apparent only when the factor of individual variation was eliminated from the calculations. During the second year 3 men from each thiamine intake group were subjected to a 3 day period of work at such a rate that 6000 calories were required for daily equilibrium. The other men were on the same work and dietary regimen for 10 days. Both experiments showed no significant change in thiamine excretion where as the pyrimin excretion increased significantly during the first 2 to 3 days, followed by a decline to below the control levels.

Some properties of  $\beta$ -keto acid carboxylases from plants. J. CATHALA (by invitation), M. C.

GOLLUB (by invitation), J. SPECK (by invitation) and B. VENNESLAND. *Department of Biochemistry, University of Chicago*. Previous observations on the occurrence of oxalacetic carboxylase in various plant preparations have been extended. The enzyme from black and red radishes, carrots and parsnip has been shown to be independent of the pyruvic carboxylase which is present in all the crude extracts from these sources. The oxalacetic carboxylase from parsley root has been subjected to a detailed study. The ability of a number of cations to activate this enzyme has been demonstrated.  $Mn^{++}$  is the best activator, but  $Cd^{++}$ ,  $Co^{++}$ ,  $Zn^{++}$ ,  $Ca^{++}$ ,  $Pb^{++}$  and  $Mg^{++}$  are also capable of showing cofactor effects. The enzyme is rapidly destroyed at room temperature on exposure to hydrogen ion activities below pH 5 or above pH 9. The active protein has been subjected to a partial purification and studies of the purified fractions with regard to their content of biotin, malic dehydrogenase and isocitric dehydrogenase are in progress and will be reported.

Repletion of protein depleted dogs with various proteins and protein hydrolysates. BACON F. CHOW and SHIRLEY DEBIASE (by invitation). *Division of Protein Chemistry, The Squibb Institute for Medical Research, New Brunswick, New Jersey*. Dogs were depleted of plasma proteins on a diet free from proteins but adequate in vitamins and calories for a period of 4 to 6 weeks. Attempts were made to replete these animals with a diet containing hydrolyzed and undigested proteins with different amino acid patterns and biological values, with a daily intake of 0.35 grams nitrogen per kgm body weight. The total circulating plasma proteins were determined before depletion, after depletion, and during repletion. Plasma albumin decreased from 45% to 25% of the total plasma protein. The results on repletion confirm our previous findings that casein hydrolysate will regenerate both plasma albumin and globulins, whereas, lactalbumin hydrolysate will regenerate mainly albumin. Supplementation of lactalbumin hydrolysate with amino acids to give a composition similar to casein hydrolysate or supplementation of casein hydrolysate with methionine to increase the nitrogen retention to that of lactalbumin hydrolysate did not alter the plasma protein regeneration properties of the original hydrolysates. Several whole protein mixtures such as egg albumin, Melactin (a mixture of casein and lactalbumin) and wheat gluten were also fed to the depleted dogs. It was found that wheat gluten did not stimulate any appreciable regeneration of plasma proteins at this level of intake. The responses of the depleted dogs to the diet of either Melactin or egg albumin, in spite of their high biological values is much slower than casein or lactalbumin hydrolysate.

The distribution of amino acids between fetal and maternal extracellular fluids and cells HALVOR N. CHRISTENSEN *The Children's Hospital, and the Department of Biological Chemistry, Harvard Medical School*. A comparison has been made of certain amino acid concentrations in fetal and maternal plasma and tissues. In the guinea pig the levels of glycine and of non glycine, non-glutamine ("residual")  $\alpha$  amino nitrogen (by ninhydrin) was about five times as high in the fetal plasma (umbilical cord blood) as in the maternal plasma. The concentrations of glycine in the muscle cells of both the fetus and mother were about nine times the concentrations in their respective plasmas and the concentrations of "residual"  $\alpha$  amino nitrogen in the muscle cells of both organisms were about 6 times the concentrations in the respective plasmas. Thus the muscle cells of the fetus contain about 4 times as high a level of amino acids as the muscle cells of the mother, due, however, to the concentrating of amino acids by the placenta rather than to an unusual ability of fetal muscle to concentrate amino acids. The administration of glycine by mouth to the pregnant animal caused a larger increase in the glycine of the fetal plasma than of the maternal plasma. The response of the concentrating ability of the placenta to the administration of amino acids was analogous to the response of the cells of liver and muscle. These observations have a bearing on the rapid protein synthesis by the fetus.

The mechanism of enzymatic synthesis of citrulline from ornithine PHILIP P. COHEN and SANTIAGO GRISOLIA (by invitation) *Department of Physiological Chemistry, University of Wisconsin Medical School*. Glutamic acid has previously been shown to play a specific role in the conversion of ornithine to citrulline in rat liver homogenates (Cohen and Hayano, *J Biol Chem*, **170**, 687, 1947). More recently it has been shown that washed residue of rat liver homogenate contains the enzyme system catalyzing this reaction (Cohen and Hayano, *J Biol Chem*, in press). Present studies on the mechanism of this reaction using washed rat liver residues supplemented with adenosine triphosphate, adenylic acid, magnesium and inorganic phosphate ions, indicate that glutamic acid acts as the acceptor for  $\text{CO}_2$  and ammonia to form an intermediate compound which reacts with ornithine to form citrulline. The fixation of  $\text{CO}_2$  and ammonia by glutamic acid requires oxygen while the transfer reaction proceeds anaerobically. Replacement of glutamic acid by glutamine, fumarate, succinate,  $\alpha$ -ketoglutarate, aspartate, oxalacetate, isocitrate and pyruvate gave the following percentage yields of citrulline, representing glutamic acid as 100 per cent: 100, 19.2, 17.6, 3.7, 0, 0, 0, 20. Attempts to demonstrate direct fixation of  $\text{CO}_2$  and ammonia

by ornithine have been unsuccessful. The nature of the intermediate compound of glutamic acid and  $\text{CO}_2$  and ammonia is at present under investigation.

The action of insulin on glycogen reserves GEORGE L. CORIENS, JR. (by invitation), EDWARD M. BRIDGI, and BARBARA MITTLAND (by invitation) *From the Stutler Research Laboratories of the Children's Hospital, Buffalo, and Department of Pediatrics of the University of Buffalo, Buffalo, New York*. The action of insulin in stimulating the deposition of muscle glycogen is well established. While numerous experiments have been reported showing that a similar stimulation to glycogen deposition does not occur in the liver, there are differences of opinion regarding the interpretation of the findings. Bouchaert and de Duve (*Physiol Rev*, **27**, 39-71, January 1947) believe that the failure of insulin preparations to stimulate glycogen deposition in the liver is due to impurities in the insulin used. Others interpret the findings as a direct effect of the hormone itself. Experiments will be presented showing the effect of insulin preparations of varying degrees of purity on liver and muscle glycogen. The effect of endogenous hyperinsulinism (pancreatic adenoma) on glycogen stores will also be demonstrated.

Changes in the composition of the blood of rats fed L-histidine H. R. CROOKSHANK (by invitation) and CLARENCE P. BERG *Biochemistry Department, Medical College of Alabama and the Biochemical Laboratory, State University of Iowa*. The course of histidine metabolism was studied in previously fasted rats by assaying the blood for histidine, total imidazole, and amino, urea, and total non protein nitrogen, in some instances before, and in others at various intervals after feeding single doses of L-histidine. All of these components but the urea attained maximal concentrations in 1 hour after the feeding. In 6 hours, free histidine had disappeared and total imidazole had returned to the fasting level. Amino and non protein nitrogen diminished fairly rapidly in this period, then more slowly until the fasting levels were attained (in 12 to 16 hours). The urea rose perceptibly in the 2nd hour, became maximal in 4 to 8 hours, then fell gradually to its initial minimum in 12 to 16 hours. The small differences noted in these tests between the concentrations of histidine and total imidazole seem to preclude any considerable conversion of L-histidine to intermediates containing the imidazole ring. On the other hand, the differences between the increases in amino nitrogen found by assay and the increases attributable to histidine seem sufficient to warrant assuming either that histidine is converted into a metabolite which retains the amino nitrogen, or that, under the stimulus of the histidine, amino nitrogen is produced from some other source. The data seem



consistent with the theory that L-histidine is converted normally, at least in part, to glutamic acid. Studies in progress involve further tests on L-histidine and similar tests on D-histidine.

**Dietary influence on the amino acid composition of proteins** FRANK A. CSONKA *From the Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, United States Department of Agriculture, Washington*. It was previously shown that the cystine and methionine content of a hen's egg may be increased by feeding a high protein casein diet (Csonka, Denton, and Ringel *J Biol Chem*, 169: 259 [1947]). Both crystalline egg albumin and conalbumin have been now prepared from individual eggs laid by hens fed different diets, and their cystine, methionine, and total nitrogen contents determined. The results show that the amino acid composition of these proteins is influenced by the hen's diet. It was also found that the nitrogen content of the crystalline albumin obtained from eggs produced by hens fed high protein diets was higher than that produced on low protein diets. The conalbumin of eggs produced by hens fed a high protein soybean diet contained a higher nitrogen content than when fed at a lower protein level. The lowest nitrogen content was observed when the eggs were produced on a high protein casein diet. The cystine and methionine percentages in both egg white proteins were not constant, and significant differences were observed depending on the dietary regimen. The cystine and methionine contents of the crystalline albumin were consistently higher in the samples prepared from eggs laid by the same hens fed a low protein diet as compared with those receiving the high protein casein diets. A significant drop in methionine percentages occurred in both egg albumin and conalbumin when the diet was changed from low to high protein soybean diet.

**Critique of methods for determination of riboflavin in urine** ELMER DE RITTER (by invitation), MARY E. MOORE (by invitation), ERICH HIRSCHBERG (by invitation), and SAUL H. RUBIN *Nutrition Laboratories, Hoffmann-La Roche, Inc., Nutley, N. J.* Riboflavin assays by several fluorimetric and microbiological procedures have been compared for human urines, ranging in content from high to vanishingly small amounts. The recent fluorimetric method of Slater and Morell (*Biochem J*, 40, 644, 1946) yielded slightly lower results for normal urines than a Florisil adsorption procedure, which, in turn, gave lower values than the double reduction method previously reported from this laboratory (*Ind Eng Chem, Anal Ed*, 17, 136, 1945). However, the differences between the Slater-Morell values and those obtained by the other fluorimetric methods were much smaller than those found by Morell and Slater (*Biochem*

*J*, 40, 652, 1946) with similar methods. This better agreement was due to the fact that the present modifications of the Florisil and double reduction procedures included a permanganate treatment in both cases and the use of a minimal amount of hydrosulfite for blank determinations in the double reduction procedure. Microbiological assays, even those calculated by the slope ratio method, were less reproducible than fluorimetric assays, ranging between the Slater-Morell and the Florisil values.

High levels of urea in association with normal riboflavin levels caused slight lowering of titrimetric microbiological values, but did not affect the turbidimetric measurements. High urea/riboflavin ratios in low-riboflavin urines, collected during total intravenous feeding of protein hydrolysates (*Fed Proc*, 6, 287, 1947), led to serious errors in titrimetric assays. For this reason, as well as for speed and convenience, turbidimetric measurement is preferred for the assay of urine.

**The breakdown of free and combined adenosine-5-phosphate (AdPh) in intact human erythrocytes** ZACHARIAS DISCHE *Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*. In washed human erythrocytes kept for 15 minutes at 37° in presence

of  $\frac{M}{40}$  NaF,  $\frac{M}{100}$  Na bromo acetate and glucose ATP disappears completely while glucose is phosphorylated to hexosediphosphate and AdPh is formed. If these erythrocytes are kept for 6 days at 0° 50-80% of AdPh disappears and simultaneously hexosediphosphate and triosephosphate are formed. Their amount accounts for about 60% of disappearing ribose. When erythrocytes are treated in the same way without addition of glucose some adenylylphosphate remains inside cells during all the time of the experiment. AdPh disappears, though less than in presence of glucose. Hexosediphosphate + triosephosphate account now for up to 100% of disappearing pentose. No significant amounts of hexosemonophosphate were found in either type of experiment. These findings suggest, that in human erythrocytes AdPh is split in triosephosphate and a dicarbon compound. The latter is synthesized in presence of ATP to hexosediphosphate. In experiments without addition of glucose the breakdown of AdPh is accompanied by its enzymatic liberation from the coenzyme fraction. This was recognized by determining free AdPh and that in coenzymes by the orcinol method according to Dische and Schwartz making readings

after 3' and 20' heating  $\frac{20' \text{ value}}{3' \text{ value}}$  is 1.22 for AdPh

and 2.25 for coenzymes. Every ribosephosphate molecule breaking down in erythrocytes seems to pass finally the stage of triosephosphate.

The effect of thyroidectomy and of thiouracil

**on cytochrome c metabolism and liver regeneration** DAVID L DRABKIN *Dept of Physiological Chemistry, Graduate School of Medicine, Univ of Pennsylvania, Philadelphia* The experiments to be reported have disclosed an apparently significant influence of the thyroid gland on the cytochrome c content of all tissues, an effect which suggests a mechanism whereby thyroxine helps regulate the oxygen consumption of tissues and the whole body

Adult rats of average starting weight of 200 gm were used, and were maintained on a vitamin supplemented, high (31%) protein diet In the completely thyroidectomized animals, liver lobectomy was performed 35 days after thyroidectomy, and the experiments were terminated 14 days later In the thiouracil studies, 50 mgm of the drug were administered daily in a measured quantity of the diet for a period of 45 days before either sacrifice or liver lobectomy Cytochrome c, water, and ribose and desoxyribose nucleic acids were determined in liver before and after regeneration, and cytochrome c and water content were measured also in kidney cortex, heart and skeletal muscle terminally Thyroidectomy induced a striking reduction in total body cytochrome c, reflected in the concentration of the pigment in individual organs and tissues The decrease in cytochrome c was about twice as great in skeletal muscle as in liver (41 against 22% reduction), with intermediate decreased values in heart and kidney cortex In the thyroidectomized rats, liver regeneration was not strikingly interfered with, although it was of smaller magnitude than normally The administration of thiouracil produced a reduction in the cytochrome c of tissues of similar magnitude as after complete thyroidectomy

**The anti-ulcer activity of aluminum dihydroxyaminoacetate.** R L DRYER (by invitation), J KATZ (by invitation), W D PAUL (by invitation), and J I ROUTH *Departments of Biochemistry and Internal Medicine, College of Medicine, State University of Iowa, Iowa City* The anti-ulcer activity of aluminum dihydroxyaminoacetate (ADA) has been investigated in rats with ligated pylorus The animals, of both sexes, ranged in weight from 150 to 300 gm and were maintained on a stock ration Prior to pyloric ligation all animals were starved 72 hr with free access to water The ligation was performed under light ether anaesthesia, recovery from this procedure was prompt and satisfactory The excised stomachs of rats given 2 cc of N/100 HCl, H<sub>2</sub>O or 0.9% NaCl by mouth immediately after the ligation consistently exhibited ulceration when examined 6 hr later A number of animals received 240 mgm of ADA in the 2 cc of HCl, H<sub>2</sub>O or NaCl Excision and examination of the stomach was performed 6 hr after the above treatment

The volume of the gastric contents, free and total acidity, pH, and peptic activity was determined in all cases Results of these experiments show that in the absence of ADA animals uniformly suffered erosion or ulceration of a more or less severe degree In this respect, no difference could be found in the nature or quantity of ulceration produced by either HCl, H<sub>2</sub>O, or NaCl In the presence of ADA the incidence of erosion and ulceration was markedly reduced No correlation could be established between degree of ulceration and either free or total acidity or peptic activity

**Dimethylthetin as a methylator of homocysteine** JACOB W DUBNOFF and HENRY BORSOOK *William G Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California* A partially purified enzyme has been isolated from the liver and kidney of the rat, guinea pig, and hog, which transfers one methyl group from dimethylthetin to homocysteine In whole liver homogenate dimethylthetin is 6 times as effective as betaine and 60 times as effective as choline in methionine formation The dimethylthetin transmethylase is distinguished from the choline transmethylase by its stability to dialysis and from the betaine transmethylase by its stability at pH 4.7

**Structural investigation of hydroxyaspergillie acid, an antibiotic substance produced by *aspergillus flavus*** JAMES D DUTCHER, O WINTERSTERNER and A E O MENZEL (by invitation) *Division of Organic Chemistry, Squibb Institute for Medical Research, New Brunswick, N J* Under certain conditions of growth, *Aspergillus flavus* produces, not aspergillie acid, C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>, but hydroxyaspergillie acid, C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>, which is much less antibacterial Subsequent to the establishment of the structure of aspergillie acid as 1-hydroxy-3,6 di-sec-butyl pyrazine 2 [J Biol Chem, 171, 321, 339 (1947)] we have investigated the structure of hydroxyaspergillie acid Both compounds are hydroxamic acids as shown by the ferric chloride color test and formation of a characteristic copper salt Hydroxyaspergillie acid can also be reduced by hydrazine to the neutral desoxyhydroxyaspergillie acid Evidence that the carbon and nitrogen skeleton is the same in both compounds was obtained by sodium and alcohol reduction to comparable piperazine derivatives The indifference of hydroxyaspergillie acid towards diazonium coupling and bromination suggested, at first, that the additional oxygen atom occupied position 5 of the pyrazine ring, but subsequent reactions failed to verify this hypothesis Hydriodic acid reduction caused the elimination of the elements of H<sub>2</sub>O with the formation of a product containing a non-cyclic double bond, and heating with strong phosphoric acid similarly led to a dehydro-product The most

likely structures therefore were those in which the additional hydroxyl group occupied the tertiary position of one or the other of the secondary butyl side chains. Confirmation of the nature of the dehydro compounds has been achieved by reduction to products identical with reduction products of aspergillie acid, but the precise location of the hydroxyl group has not been established yet. However, the formulation which places it in the side chain situated at position 6 is favored.

**Measurement and study of immune precipitates by means of ultraviolet absorption spectroscopy** HERMAN N EISEN and ALBERT S KESTON (introduced by ROBERT C WARNER) *Departments of Medicine and Chemistry, New York University College of Medicine* Ultraviolet absorption spectroscopy of specific precipitates furnishes an accurate, sensitive method for the quantitative measurement of serum antibody levels. The capsular polysaccharide of pneumococcus type V and rabbit pneumococcus type V antiserum gave a precipitate whose absorption spectrum was that of the antibody protein. This spectrum was found not to change over a broad range of the precipitin curve. However, when a protein antigen (bovine serum albumin) was used with homologous rabbit antiserum, the immune precipitates showed changes in the absorption spectra in different regions of the precipitin curve consistent with the expected variations in the antigen antibody composition of the precipitates. Analysis of spectral data offers, within limits, an opportunity to determine the composition of those immune precipitates whose components have significant and different absorption spectra.

**Immunochemical studies with proteins labelled with trace amounts of radioactive iodine** HERMAN N EISEN and ALBERT S KESTON (introduced by ROBERT C WARNER) *Departments of Medicine and Chemistry, New York University College of Medicine* Proteins were labelled with  $I^{131}$  by direct iodination. The amount of iodine involved was extremely small yet highly radioactive preparations were obtained. In these preparations only an occasional protein molecule was tagged with radioactive iodine. The label was stable, and antisera against the native protein seemed unable to distinguish between labeled and unlabeled protein. Labelling of protein was also carried out by coupling with  $p$ - $I^{131}$  phenyldiazonium chloride. The preparations so obtained also retained specificity. However, part of the label dissociated from the protein over a period of weeks. Both types of labelled protein proved capable of being used as sensitive indicators in the determination of serum precipitins.

**Metabolism of cerebral cortex from different areas and various animals and from epileptic patients** K A C LLIOTT *Montreal Neurological*

*Institute, McGill University* The  $O_2$  uptake rate of slices of cerebral cortex from various animals ranging in size from mouse to man is roughly inversely proportional to the tenth root of the common body weight of the individual of the species. Though the rate varies with the composition of the suspending medium, the same relation holds in different media. Within a species, the rate does not vary greatly or consistently with the size of the individual. Slices from different areas of the cortex respire at appreciably different rates, particularly dorsal areas respire more actively than lateral in all animals studied. Usually the second slice respire somewhat more rapidly than the surface slice. Active aerobic glycolysis, usually falling off fairly rapidly, occurs in brain slices from all species if the metabolism is studied immediately after introduction of the slices into ringer bicarbonate medium. With isotonic suspensions aerobic glycolysis is not appreciable.

The relative rates of oxygen uptake, carbon dioxide evolution, aerobic glycolysis, and anaerobic glycolysis in the presence of the optimal amount of pyruvate, were the same in isotonic suspensions and slices from all the normal animals studied. Focal epileptogenic human brain tissue, excised for therapeutic purposes by Dr Wilder Penfield, respired, on the average, at the rate expected from the results on normal animals. Relatively high anaerobic glycolysis occurred with suspensions of all the human samples but it is not certain whether this is related to the epileptic process.

**The quantitative estimation of steroid alcohols** LEWIS L ENGEL, HELEN R PATTERSON and HILDEGARD WILSON (introduced by A BAIRD HASTINGS) *Medical Laboratories of the Collis P Huntington Memorial Hospital of Harvard University, at the Massachusetts General Hospital, Boston, Massachusetts and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts* Studies of the intermediary metabolism of the steroid hormones have been hampered by the absence of a simple and relatively specific method for the estimation of steroid alcohols in urinary extracts. In an effort to devise such a method it was found that hemi-3,5 dinitrophthalates of steroid alcohols develop a color suitable for analytical purposes upon treatment with methanolic potassium hydroxide. Pure steroid alcohols were treated with excess 3,5 dinitrophthalic anhydride in pyridine solution. After dilution of the reaction mixture with hydrochloric acid the half esters which were quantitatively formed were extracted with ether, washed and transferred to methanol. For analysis, aliquots were diluted with methanol and 5N methanolic potassium hydroxide so that the final potassium hydroxide concentration was 1.5N. The color developed showed maximal absorption at 510 m $\mu$ .

and adherence to Beer's Law in the range 0.3-15  $\mu$  M of ester, provided readings were made 5-15 minutes after mixing the reagents. At higher alkali concentrations sensitivity was increased but more rapid fading was encountered. No significant color was developed by 3,5 dinitrophenolic acid in 1.5 N methanolic potassium hydroxide. The absorption spectra, extinction coefficients and variation of extinction coefficients with alkali concentration were similar for all of the compounds thus far studied. Preliminary results indicate that this method may be applied to the estimation of alcohols in urinary extracts.

**Influence of polyhydric alcohols on the clotting of fibrinogen** JOHN D. FERRY and SIDNEY SHULMAN (by invitation) *Department of Chemistry, Univ. of Wisconsin*. When a fibrin clot is formed from solutions of bovine fibrinogen and thrombin, its properties and structure depend markedly (as in the case of human fibrin) on the pH, ionic strength, and other conditions. At pH 6.3 in 0.15 M NaCl, a "course" clot (opaque, readily synergizing) is formed. The coarseness of structure, as measured by opacity, is profoundly affected by small quantities (1 to 5%) of hydroxyl compounds. Monohydric alcohols (ethyl, butyl) increase opacity slightly, compounds with 2 to 6 hydroxyls decrease opacity, compounds with many hydroxyls (polyvinyl alcohol, starch) increase opacity. The concentrations in moles OH per liter required to decrease clot opacity to a given value provide a measure of comparative effectiveness: glycerol and mannitol, 1.6; 2-methyl-2,1-pentanediol and pentaerythritol, 0.5; tetramethylene glycol, 0.25; hexamethylene glycol, 0.2; pentamethylene glycol, 0.1. All of these substances prolong the clotting time. They do not affect thrombin activity. They have no irreversible effect on the fibrinogen, when introduced and then removed by dialysis before clotting; they cause no alteration of clot structure.

These results indicate an interaction between fibrinogen and polyhydric alcohols which depends critically on the spacing between hydroxyl groups. The interaction increases in the order  $0 < 1 < 2 < 4 < 3$ , where the numbers denote the number of carbon atoms between carbons bearing hydroxyls.

**The quinoid stage of tyrosine metabolism** ELIA H. FISHBERG *Biochemical Laboratory of Belk Israel Hospital, New York City*. It has recently been shown that benzoquinone acetic acid, a substance capable of *in vitro* and *in vivo* met-hemoglobin formation in subjects showing evidence of vitamin-C depletion. It is an obligate intermediate in the normal catabolism of tyrosine and its existence in the blood can be demonstrated by a characteristic absorption spectrum in the ultraviolet. In certain subjects where the tyrosine metabolism is interrupted at the quinoid stage, the amount of benzoquinone acetic acid excreted in

the urine can be determined quantitatively by titrating with thiosulfate the amount of I released by the quinone from an acidified solution of KI. Using this value in conjunction with the density of the u.v. absorption spectrum of this urine it becomes possible by appropriate dilution to determine the concentration of benzoquinone acetic acid in the blood. The concentration of BQA in the blood is constant in the normal organism. We have encountered marked deviations only in those conditions in which some abnormality of liver function may be clinically demonstrated or experimentally induced.

**Blood plasma anti-glucuronidase activity** WILLIAM H. FISHMAN, KURT I. AITMAN (by invitation) and BEILI STRINGER (by invitation) *Departments of Surgery and Biochemistry, University of Chicago, Chicago, Ill.*  $\beta$ -Glucuronidase activity is strongly inhibited in the presence of untreated or boiled mammalian blood plasma or serum. Only a slight decline in inhibitory activity is observed with plasma dilutions of 1 to 5 or 10. With greater plasma dilutions the inhibitory activity diminishes more markedly, although demonstrable effects are still evident at 1 to 100 dilution. The activity of several other enzymes is not affected by boiled plasma suggesting a measure of specificity to the glucuronidase inhibiting principle. Body tissues contain very much less glucuronidase inhibitor activity than blood. Very frequently also, glucuronidase activity is inhibited in the presence of urine although here much of the inhibition disappears after dialysis. In plasma, glucuronidase inhibiting power is only slightly affected by dialysis either before or after boiling. However, when acidified plasma is boiled, the inhibiting activity is removed with the precipitated protein. From these observations it would appear that plasma glucuronidase inhibition may be a property of a large, heat stable, non-dialyzable molecule. It is proposed that the glucuronidase inhibition of blood plasma may be due to an anti-enzyme which possibly may play a role in the regulation of tissue glucuronidase activity.

**Alkaline phosphatase activity of intestinal lymph of the rat** EUNICE V. FLOCK and JESSE L. BOLLMAN *Mayo Foundation, Rochester, Minnesota*. The intestinal mucosa has been considered as a possible source of the alkaline phosphatase of plasma largely owing to its high content of phosphatase and the observation of a decrease in plasma phosphatase following fasting and an increase after certain meals. Analysis of the intestinal lymph provides more direct evidence for this function of the intestinal mucosa.

The intestinal lymphatics of rats were cannulated with polyvinyl plastic tubing. A control sample of lymph was collected overnight, a meal given by stomach tube, and the lymph collected

continuously. Analysis of the lymph collected was made at the 2nd, 4th, 6th, 12th, and 24th hours. Following a fat-free meal the alkaline phosphatase activity of the lymph increased 3 to 6 times the control values by the 6th hour and remained high through the 12th hour after which the activity returned to the original value. Following a similar meal to which fat had been added the phosphatase activity was similar to that obtained with the fat-free meal alone. The phosphatase activity of plasma collected from fed rats after 2 days of lymph drainage was as low as that in normal rats fasted for this period of time.

**Brain proteins: isolation of a birefringent liponucleoprotein.** JORDI FOLCH and L. L. UZMAN (by invitation). *McLean Hospital, Waterbury, Mass., and Harvard Medical School.* Brain tissue is treated with a 4:1 mixture of ether  $\text{CH}_3\text{OH}$  and then with ether at  $-15^\circ$ . The residue is extracted with water which is changed every few hours. Extracts are cleared by 12,000 rpm centrifugation, brought to pH 8, then to 4.5, precipitate (A) that forms collected, and the filtrate  $\frac{2}{3}$  saturated with  $(\text{NH}_4)_2\text{SO}_4$ . Any precipitate that forms is dialyzed and lyophilized (B). Material B is obtained only during the first 12 hours of extraction while A is obtained even after 48 hours. B is a protein, free of P and lipids. A is a liponucleoprotein. It is insoluble at pH 4.5 and soluble at higher and lower pH. It contains 35 per cent lipids and 1.1 per cent P. Lipids are firmly bound (prolonged boiling with  $\text{CH}_3\text{OH}$ ,  $\text{CHCl}_3$  being required for their removal) and are found to be a mixture of phosphatides and carbohydrate containing lipids. The lipid free material contains 0.72 per cent P (it accounts for half of the brain protein P) and purines with the  $\frac{\text{purine}}{\text{P}}$  ratio of a nucleoprotein. Ultraviolet absorp-

tion spectrum of a solution of the isolated purines is almost identical with that of an equimolar solution of guanine and adenine of same concentration. The liponucleoprotein is birefringent and its X-ray pattern (Dr. Bear, MIT) shows a degree of order consistent with crystalline structure.

**Fractionation of serum cholesterol.** J. C. FORBES and WILLIAM B. PORTER (by invitation). *Depts. of Biochemistry and Medicine, Medical College of Virginia, Richmond, Virginia.* Experimental data will be presented showing that when normal blood serum, dried in vacuo from a frozen state, is extracted with chloroform at about  $5^\circ\text{C}$ , a small but constant percentage of the total cholesterol is extracted. This fraction in the case of 20 normal individuals varied from around 7 to 18%, the majority being between 10 and 13%. On the other hand the extractable cholesterol of the serum of patients with the nephrotic syndrome with marked hypercholesterolemia was very high, being in some cases well over 90%. Data will also be presented on other

clinical conditions as well as on rabbits rendered hypercholesterolemic by means of a high cholesterol diet.

**Concerning the mechanism of interaction of egg white trypsin inhibitor and trypsin.** HEINZ FRAENKEL CONRAT, R. S. BEAN (by invitation), and HANS LINEWEAVER. *Western Regional Research Laboratory, Albany, Calif. Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.* The trypsin inhibitor of egg white was recently identified as native ovomucoid (Hans Lineweaver and C. W. Murray, *J. Biol. Chem.*, in press). Unpublished results from this Laboratory show that, in contrast to other trypsin inhibitors, ovomucoid has weak to no action on other proteolytic enzymes. Because of this specificity, the mode of interaction of trypsin and ovomucoid appears of particular interest. The chemistry of this interaction has been studied through the preparation of derivatives of both proteins.

The main results were: (a) Acetylation of most of the amino groups inactivated neither the inhibitor nor trypsin. But acetylated trypsin was no longer susceptible to inhibition by ovomucoid or its acetyl derivative. (b) In contrast to amino groups, most carboxyl, phenolic, guanidyl, and hydroxyl groups in ovomucoid appeared essential for its inhibiting action. (c) The proteolytic activity of trypsin was independent of its most reactive carboxyl and phenolic groups, but depended upon the integrity of amide, guanidyl, hydroxyl, and indole groups. Thus it appears that trypsin combines with the substrate through groups other than amino, but combines through its amino groups with the acid groups of the inhibitor. Other groups in both proteins probably perform important secondary functions in stabilizing the primary ionic combination. Limited kinetic studies indicate that inhibition of trypsin by ovomucoid is of the noncompetitive type, in agreement with the chemical indications that the substrate and inhibitor combine with different active groups of trypsin.

**Oxidation-coupled phosphate exchanges in the acid-insoluble esters of cell-free liver preparations.** MORRIS E. FRIEDMAN (by invitation) and ALBERT L. LEHNINGER. *Departments of Biochemistry and Surgery, University of Chicago, Chicago.* Esterification of inorganic phosphate coupled to the oxidation of malate takes place in properly supplemented suspensions of particulate material from rat liver, as demonstrated by rapid incorporation of inorganic phosphate (labelled with  $\text{P}^{32}$ ) into the acid soluble esterified fraction. In order to determine whether the oxidation coupled esterification of phosphate in the acid-soluble fraction is functionally related to phosphate exchanges in the acid-insoluble esters, we have studied  $\text{P}^{32}$  distribution in the latter following

aerobic incubation of the suspensions in the presence of 1 malate, ATP,  $Mg^{++}$ , KCl, inorganic phosphate labelled with  $P_3$ , and glycylglycine buffer. After extraction of acid soluble phosphate the acid-insoluble P was separated into phospholipid, nucleic acid and an unidentified P containing residue ("phosphoprotein") by the method of Schneider (J Biol Chem, 161, 293 (1951)). The nucleic acid fraction was further separated into ribo- and desoxyribo nucleic acid fractions. The specific radioactivity of each fraction was determined. The acid insoluble P showed a much lower rate of turnover than the acid soluble P, comparable to that observed in the intact liver. All three acid-insoluble P fractions showed incorporation of  $P^{32}$ . Desoxyribonucleic acid showed no significant radioactivity although the two nucleic acids are present in roughly equal amounts in the suspension. The high specific activity of the "phosphoprotein" fraction suggests an active metabolic role for the unidentified esters present.

**Conversion of protein to glucose in vitamin deficiencies.** OLIVER H. GABELLER and PAUL BARTLETT (by invitation) *Henry Ford Hospital, Detroit*. The relationship between glucosuria and vitamin deficiency in experimental pancreatic diabetes may be complicated by the fact that interference with formation of glycogen or fat, or with oxidation, would increase glucosuria, while slower absorption or diminished glucogenesis from protein would decrease it (Endocrinology 39:239, 1946). To study glucogenesis from protein separately, D/N ratios were determined in dogs during various deficiencies. The animals received a purified, carbohydrate-free diet high in vitamin-free casein, and were phlorhizinized after depletion of one vitamin. D/N ratios were determined for 4 days during the deficiency, and for several days after supplying the missing vitamin. Ratios before and after deficiency could thus be compared in the same animal. Two dogs depleted of thiamine for 25 and 27 days respectively showed average ratios of 3.26 and 3.27, after terminating the deficiency the average ratios were 3.34 and 3.51. In dogs depleted of riboflavin for 51 and 55 days, the average ratios were 3.26 and 2.95, after administering this vitamin the average ratio in the first animal was 3.13. Results in pyridoxine deficiency have been reported in another connection (Fed Proc 6:236, 1947). In all experiments the characteristic effects of deficiency were present and responded dramatically to administration of the missing vitamin. None of the ratios observed during deficiencies were strikingly different from values obtained with casein by other observers, and the increases in the ratios after terminating deficiencies of thiamine and pyridoxine were not decisive.

**Studies on glycolysis of mouse lymphosarcoma.** HELENA GILDER (introduced by VINCENT DU

VIGNAUD), MARION H. WILSON (by invitation), JOHANNA M. LEFF (by invitation) *Department of Biochemistry, Cornell University Medical College, New York, N. Y.* Aerobic and anaerobic glycolysis of the Gardner lymphosarcoma cells have been studied in the Warburg manometer. As is characteristic of tumor tissue the lymphosarcoma cells have a high aerobic and anaerobic glycolysis when optimal glucose and bicarbonate concentrations are present. Comparative studies of the effectiveness of other substrates in lieu of glucose suggest that the metabolic pathway for lactic acid production in the cells studied may not be identical with the Priden-Meyerhof-Cori metabolic pathway demonstrated in muscle and in yeast. These studies indicate substantially decreased glycolysis when certain phosphorylated carbohydrates are substituted for glucose.

**Fractionation of lymphoid tissues.** LRLAND C. GIESING (introduced by ALFRED CHANTIN) *Biochemical Laboratory, University of Virginia*. Calf thymus and mouse thymomas were fractionated by utilizing the low temperature ethanol procedures introduced by Cohn and associates. The stroma, lymphocytes and the saline soluble components were first separated and each was subjected to fractionation. The nucleic acid distribution for the various fractions was determined by spectrophotometric and chemical analyses. A nucleic protein-rich fraction was obtained from the stroma. Three fractions were obtained from lymphocyte nuclei, two distinctly different nucleic proteins and a nucleic acid-free protein were precipitated. The saline soluble components were separated into 4 fractions. They were characterized as follows. The largest fraction obtained by iso-electric precipitation contained nucleic acid, two fractions were free of nucleic acid and the remaining precipitate was rich in lipides. Using the same fractionating procedures of thymus and thymomas, a number of differences in the types of precipitates were noted. These phenomena will be discussed.

**Rate of  $C^{14}O_2$  excretion following intraperitoneal administration of isotopic bicarbonate and acetate.** R. GORDON GOULD, (by invitation), I. M. ROSENBERG, (by invitation), MAROTT SINEX, (by invitation), and A. BAIRD HASTINGS *Dept of Biological Chemistry, Harvard Medical School, Boston, Mass.* Fasted rats were injected with  $C^{14}$  labeled bicarbonate in amounts of less than one milligram and the respiratory excretion of  $C^{14}O_2$  followed for 4-6 hours. The results permit calculation of the specific activity of respiratory  $CO_2$  (and therefore of blood bicarbonate) and its variation with time, as well as the extent of excretion of the  $C^{14}$ . The specific activity of respiratory  $CO_2$  reached a maximum value within 10 minutes after injection and then decreased exponentially for approximately an hour, at a rate corresponding

to a decrease in value of 50% in 16 minutes. At about 80-100 minutes the rate of decrease changed to a considerably slower one. Carboxyl-labeled acetate of high specific activity was administered in small dosage in the same manner and half of the  $C^{14}$  was recovered as respiratory  $CO$  within 40-50 minutes. The specific activity attained its maximum value in 15-25 minutes, it then decreased exponentially during the subsequent 2 hours, but at a slower rate than the initial rate observed following isotopic bicarbonate administration. Carboxyl labeled succinate was also studied and the results, which were in contrast to those obtained with acetate, will be presented. The extent of excretion during the experimental period was over 90% for bicarbonate and somewhat less for acetate.

**Xanthurenic acid excretion in pyridoxine deficient rhesus monkeys** LOUIS D. GREENBERG and JAMES F. RINEHART (by invitation) *Divisions of Pathology and Pharmacology, University of California Medical School, San Francisco*. Using the method of Miller and Baumann (*J Biol Chem* 157, 551, 1946) the quantitative excretion of xanthurenic acid was followed in 7 pyridoxine deficient rhesus monkeys on the diet and supplements previously described (*Fed Proc* 5 222, 1946). Estimations of the xanthurenic acid eliminated in urine were made before and after the administration of 0.5-1.0 gram doses of DL- or L-tryptophane per os. Four animals were studied in this manner before and after withdrawal of vitamin  $B_6$  from the diet, so that they could serve as their own controls. On a good intake of the vitamin only small quantities (apparent xanthurenic acid 1.5-3.0 mg) of substances reacting with the  $FeCl_3$  reagent were excreted before or after the administration of tryptophane. These values probably represent compounds other than xanthurenic acid since this reagent reacts with phenylpyruvic acid and most likely with other  $\alpha$ -keto acids. As early as 18 days after withholding pyridoxine, there occurred marked increases in the xanthurenic acid excretion following the feeding of tryptophane. However, only a small portion of the total test dose of tryptophane was excreted as the chromogenic compound, for example, the average excretion expressed as per cent of the test dose is 2.1 with 0.5 gram DL-, 3.0 with 1.0 gram DL-, and 3.3 with 0.5 gram L-tryptophane. The urine specimens of moderately or severely deficient monkeys were usually colored green and the intensity of the color was increased by the addition of ferric chloride.

**Partial purification of cathepsin II from swine kidney** HELMUT R. GUTMAN (by invitation) and JOSEPH S. FRUTON. *Dept of Physiological Chemistry, Yale University New Haven*. Aqueous extracts of swine kidney contain a wide variety of proteolytic enzymes. In addition to the endo-

peptidase (cathepsin II) which hydrolyzes benzoyl-L-argininamide, there are present several exo and endopeptidases. In the crude extract, the exopeptidase activity toward substrates such as glycylglycylglycine, L-leucinamide, or carbobenzyloxylglycyl-L-phenylalanine exceeds the activity of Cathepsin II. A purification procedure has been devised for Cathepsin II which permits the elimination of the known exopeptidases and the other endopeptidases for which synthetic substrates are available. This procedure involves fractional precipitation with ammonium sulfate, dialysis against distilled water, precipitation with yeast nucleic acid, selective extraction of the protein nucleate, and finally, fractional precipitation with ammonium sulfate. The purified enzyme hydrolyzes, in addition to benzoyl-L-argininamide, gelatin, denatured hemoglobin, and salmine. As in the case of the synthetic substrate, the digestion of the proteins is favored by the presence of cysteine. When purified cathepsin II is partially inactivated by heat, the rate of cleavage of benzoyl-L-argininamide, gelatin, and salmine is decreased to about the same extent. This result supports the view that the endopeptidase activity of Cathepsin II is associated with a protein splitting enzyme.

**Further observations concerning antithiamine activity in plants** J. R. HAIG and P. H. WESWIG (by invitation) *Oregon Agricultural Experiment Station, Corvallis, Oregon*. Our previously announced studies (*Journal of Biological Chemistry*, 165, 737 and *Federation Proceedings*, 6, 408) dealing with thiamine deficiency induced in rats fed rations containing air dried bracken fern have been continued and extended to related plant species. Bracken fern rations, compounded from a single lot of fern showed decreasing toxicities in the order of fresh fern, air dried fern fed in moist rations, and air and sun dried fern. We have been unable to demonstrate any harmful effects to rats of rations containing as much as 40% of their dry matter as fresh fern steamed for 30 minutes. Although some evidence of antithiamine activity in related plants has been obtained, none so far tested have shown activity to equal that of bracken fern. Chemical and microbiological assay procedures have raised numerous questions concerning their interpretation in terms of animal performance. The Melnick and Field procedure, as applied to NaCl extracts, seems to respond to a quick acting factor which possesses considerable heat stability. The thiochrome and microbiological procedure have so far offered more promise of results directly applicable to animals.

**An infrared and X-ray study of polymorphism in 5-pregnen-3( $\beta$ )-OL-20-one** WILLIAM J. HAINES, MARI P. GOODWIN, GEORGE PISH and FOIL A. MILLER (introduced by MARVIN H. KUTZLECA) *Research Laboratories, The Upjohn Company,*

Kalamazoo, Michigan, and Division of Physical Chemistry, University of Illinois, Urbana. A preliminary report from this laboratory (paper presented at Meeting of American Chemical Society, New York City, 1947) has described the isolation of 5 pregnen-3( $\beta$ )ol-20-one (pregnenolone) from hog testes. Although this natural product was readily identified by its melting point, optical rotation and the properties of its oxime derivative, unexpected differences were observed when its X-ray powder pattern and infrared absorption spectrum (Nujol mull) were compared with those obtained for a sample of synthetic pregnenolone. However, the infrared spectra of chloroform solutions of the two samples were identical. This behavior suggested the existence of polymorphic crystals. The present study was undertaken in an attempt to produce a sample of synthetic pregnenolone which had a crystal form identical with that of the natural product. Since the latter had been prepared by chromatography over magnesium silicate-"Celite" mixtures followed by crystallization from absolute methanol, these techniques were applied to a sample of synthetic pregnenolone having X-ray pattern A and infrared mull spectrum I. The first chromatogram caused a conversion to an intermediate form, X-ray pattern B and infrared mull spectrum II. Rechromatography of this material resulted in a form having X-ray pattern C and infrared mull spectrum III. This fraction was spectroscopically identical with the natural pregnenolone. The melting points, optical rotations and infrared spectra *in solution* indicated the chemical identity of these three crystal modifications.

The interrelationship of these crystal forms and their mode of interconversion are considered. The role of polymorphism in the identification of crystalline compounds is discussed.

**The metabolism of parenterally administered amino acids. I. glycine.** PHILIP HANDLER, HENRY KAMIN (by invitation) and JEROME S. HARRIS (by invitation). *Departments of Biochemistry and Pediatrics, Duke University School of Medicine, Durham, N. C.* Glycine was administered intravenously to female dogs, under dial anesthesia, at rates from 1 to 8 mg N/kilo/min. Urine was collected constantly by catheter and blood samples obtained periodically. All dogs died, in respiratory and renal failure, after 600-900 mg N/kilo had been administered; their survival time was roughly inversely proportional to the rate of administration. Samples of various organs were then removed, and frozen in acetone-CO<sub>2</sub>. Other dogs, which received 0.4% NaCl or 0.15% Na<sub>2</sub>SO<sub>4</sub> solutions for equal periods, served as controls.

The "rate of utilization" was defined as  $\alpha$  amino N administered — [ $\alpha$  amino N excreted +  $\alpha$  amino N in extracellular fluid] /kilo/min and included

cellular accumulation as well as oxidation, etc. The maximum rate of glycine "utilization" was found to be 2 mg N/kilo/min while the maximum rate of urea formation under these conditions was 1.2 mg N/kilo/min. Accumulation of  $\alpha$  amino N was most marked in liver and progressively less in kidney, heart and skeletal muscle. This was entirely glycine accumulation since, in liver, there simultaneously occurred a drop of 50-75% in the concentration of other amino acids measured by microbiological assay in tungstate filtrates. The blood concentration of these amino acids remained relatively constant, but their urinary excretion increased 5-75 fold when the blood glycine concentration was markedly elevated. However, this excretion accounted for no more than 20% of the amino acids which had disappeared from liver. When glycine was administered at 1 mg N/kilo/min, for 18 hours, urea N formation exceeded glycine N administration by about 20%. This phenomenon and the disappearance of liver amino acids are thought to be related to the specific dynamic action of glycine.

**Conditions necessary for complete decarboxylation of pure L-lysine and L-tyrosine by amino acid decarboxylases.** MERRILL E. HANKE. *Department of Biochemistry, University of Chicago.* Precise study in the Van Slyke-Nall manometric apparatus of the yield of CO<sub>2</sub> obtained from pure L-lysine or L-tyrosine with decarboxylases prepared from *Bacterium cadaveris* or *Streptococcus fecalis* respectively, has shown only 97 to 98% of theoretical. This yield of CO<sub>2</sub> is increased by removal of O<sub>2</sub> from the reaction mixture, or more conveniently and effectively, by the addition of a large excess of L-leucine or DL-alanine. Glycine or cysteine alone is entirely ineffective although cysteine does augment the effect of leucine or alanine. With 1000 micromols of DL-alanine plus 125 micromols of cysteine in 6.5 ml of reaction mixture, the yield of CO<sub>2</sub> from 5 to 20 micromols of L-lysine or L-tyrosine is 99.5%.

Our present interpretation is that the decarboxylase preparations (crude extracts of bacterial cells) contain some L-amino acid oxidase, which oxidizes the L-lysine or L-tyrosine to a slight extent (2 to 3%) before decarboxylation is complete. The leucine and alanine may act by preferential reaction with the oxidase and consequent removal of O<sub>2</sub>, or possibly by competitive prevention of access of the lysine and tyrosine to the oxidase. Added leucine or alanine with or without cysteine has no effect on the decarboxylase-CO<sub>2</sub> values of protein hydrolysates, since here there is an excess of other amino acids to tie up the oxidase. Decarboxylase CO<sub>2</sub> values for lysine and tyrosine on hydrolysates of crystalline bovine blood albumin agree to within 1 part in 200 with those reported by the isotope dilution method.



The effect of protein quality of previous intake on the consequences of acute starvation ROBERT A HARTE (by invitation), JOHN J TRAVERS (by invitation) and PETER SARICH (by invitation) (by JAMES B ALLISON) *Research Laboratories, The Arlington Chemical Company, Yonkers, 1, N Y* In animal feeding experiments the common impression is that body weights are intrinsically more accurate than food intake figures A review of physiological considerations quickly belies this impression An exploratory experiment revealed that adult rats on a stock diet lost no appreciable amount of weight during the first 2 hr following the removal of food (In all experiments there was free access to water) Thereafter weight loss was precipitous during the next 22 hr The course of weight change during subsequent refeeding and food deprivation periods was followed These observations prompted examination of the role of protein quality during the period before food withdrawal A group of 135 rats whose growth had been followed for 5 wk after weaning on synthetic isocaloric rations containing 10% (N x 6.25) of 7 different proteins were available for this study They were weighed at intervals during 24 hr following removal of food Since they had attained differing weights during the growth period the data were analyzed by covariance techniques to make approximate correction for this fact In this way the effects of quality of different proteins previously ingested on the ability of the rat to withstand short time acute starvation could be assessed When so analyzed the differences between proteins were highly significant ( $p < 0.001$ ) The proteins could be divided into 2 groups, 1 showing an average adjusted weight loss of about 7% and including whole egg, beef, egg white, and 2 casein samples, the other showing an average adjusted weight loss of about 10% and including wheat gluten and peanut flour

Action of peptidases on  $\beta$ -alanine peptides H THEO HANSON (by invitation) and EMIL L SMITH *Laboratory for the Study of Hereditary and Metabolic Disorders and the Depts of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City, Utah* Some years ago, Abderhalden and co workers reported that peptides containing  $\beta$  amino acids were not hydrolyzed by extracts of pancreas or intestine We have found that certain peptidases will attack  $\beta$  alanine peptides Prolidase of rabbit muscle hydrolyzes glycyl L proline (GP) about 200 times as fast as  $\beta$  alanyl L proline (BP) Moreover, the hydrolysis of GP is inhibited about 50% in the presence of an equimolar concentration of BP, indicating that both compounds combine with the active groups of the same enzyme Leucinaminopeptidase hydrolyzes L leucyl  $\beta$  alanine about one fourth as rapidly as it does L leucylglycine, the rates are not additive in the presence of both substrates,

and the hydrolysis of both is activated by  $Mn^{++}$  The enzyme which attacks glycylglycylglycine and other tripeptides has been found to hydrolyze glycyl  $\beta$  alanylglycine quite rapidly, glycylglycyl- $\beta$  alanine somewhat more slowly and  $\beta$  alanyl-glycylglycine extremely slowly The hydrolysis of glycyl L leucine is about 200 times as fast as that of  $\beta$  alanyl L leucine It is evident from our results with these and other  $\beta$  alanine containing substrates that the specificity of peptidases towards  $\alpha$  amino acids is not absolute The hydrolysis of compounds of unknown structure by proteolytic enzymes can no longer be regarded as proof that the compound contains only  $\alpha$  amino acids

Enzymatic cleavage of organic halides LEON A HEPPEL and VIRGINIA T PORTERFIELD (introduced by B L HORECKER) *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland* It is known that inorganic bromide accumulates in the tissues of animals given various aliphatic bromine containing compounds No *in vitro* studies are recorded In the present investigation it was found that protein fractions of the livers of several animal species catalyzed the dehalogenation of a series of aliphatic halides These included mono bromo mono chloromethane, 1,2-dibromoethane, ethyl bromide, ethylidene bromide,  $\beta$  bromopropionic acid, 1-chloro, 2-bromoethane and 1,2 dichloroethane The enzymatic activity was measured either by the determination of inorganic halide or by following the rate of  $CO_2$  evolution in a bicarbonate buffer due to the formation of fixed acid Heating for 5 minutes at  $60^\circ C$  or introducing  $10^{-3}M$   $Hg^{++}$  caused complete inhibition A five fold purification of the crude tissue extract was obtained by fractionation with ammonium sulfate Dehalogenation of mono bromo mono chloromethane resulted in the formation of formaldehyde which was determined quantitatively by the chromotropic acid method In addition, the crystalline dimedone derivative of formaldehyde was isolated and its melting point determined

The rates of absorption of l- and dl-methionine W C HESS *Georgetown Medical School, Washington, D C* In 1933 Chase and Lewis found that the rate of absorption of dl methionine from the gastrointestinal tract of the white rat was 53 mg per 100 gm of body weight per hour The method they employed to estimate the methionine remaining in the G I tract was the determination of amino nitrogen The present work details the results of feeding both dl and l methionine to white rats and measuring the rate of absorption by two methods First, the methionine remaining in the G I tract was determined by a modified McCarthy Sullivan method and second, the total sulfur content of the tract was determined By the colori-

metric method the rates of absorption were 10.0 mg and 36.8 mg per 100 gm per hour for dl- and l-methionine respectively. From the determination of the total sulfur the calculated rates were 39.1 mg and 35.4 mg per 100 gm per hour for dl- and l-methionine respectively. Both methionine and homocysteine were found in the livers of the rats fed methionine, there was no increase in the cystine or glutathione contents of the liver.

**Manganese and thiamine in the diet of mother rats and body temperature control in the young** ROBERT M. HILL and DORIS E. HORTSMAN (by invitation) *Department of Biochemistry, University of Colorado Medical Center*. A recent paper from this laboratory (Am J Physiol 119, 650-656, 1947) presented evidence that nutritional factors in the diet of the mother may modify the time of development of body temperature control against cold in young white rats. We can now report that the manganous ion is a promoting factor and that thiamine has an antagonistic action. The activity of each factor is most striking at low dietary levels of the other. In the first experiment Purina Fox Chow (700 gamma Mn and 30 gamma thiamin per day per mother rat) was used without supplement. The mothers were on this diet from the time of breeding until after the young were 18 days old. At that time the young were exposed in individual cages in the cold room at 3-6°C. No control of body-temperature was exhibited by these animals. In the second experiment 1000 gamma Mn was added to the diet per mother rat per day. At 18 days of age, one third of the young had acquired almost complete body-temperature control against cold. In the third experiment, in addition to 1000 gamma Mn, 100 gamma thiamin was added per mother rat per day. On the 18th day of life none of the young of this group showed complete body-temperature control against cold, though most of them had developed some degree of resistance. This work was repeated using powdered whole milk as the basic diet. Similar results were obtained.

**Stereochemistry of allopregnanetriol-3,16/20 of mare's urine** H. HIRSCHMANN, FRIEDA B. HIRSCHMANN (by invitation), and MARGARET A. DAUS (by invitation) *Department of Medicine, Western Reserve University and the Lakeside Hospital, Cleveland, Ohio*. Reduction with platinum in alcohol of the known 20-acetoxyallogregnanedione-3,16 derived from the allopregnanetriol 3,16,20 (I) of mare's pregnancy urine yields 20-acetoxyallopregnanol 3( $\beta$ )-one-16 (m.p. 191.5-193°) which can be acetylated to a diacetate (II) (m.p. 184-186°). The same compound has been prepared by a second route. Partial solvolysis of the triacetate of I furnishes two diacetates melting at 165-166° and at 115-116.5°. The lower melting of these on oxidation with chromic acid is converted into II. Reduction of II with platinum in acetic acid or

with nickel in alcohol followed by acetylation yielded a triacetate (III) identical with that prepared from pseudotigogenin diacetate by the method of Marker. It follows that triol I has erroneously been assigned the  $\alpha$  configuration at C-3 and that like tigogenin it is a  $\beta$ -3-hydroxy steroid. Compound III has a higher rotation ( $[\alpha]_D^{25} = +16^\circ$ ) than the 20-epimeric triacetate ( $[\alpha]_D^{25} = -25^\circ$ ) prepared from tigogenin by means of persulfuric acid. The 16-acetoxy group of the triacetate of I is solvolyzed faster than that of III which indicates that the 16-hydroxyl group of I is *trans* to the side chain at C-17.

**2-Aminopurine as a purine antagonist** GEORGE H. HITCHINGS, GERTRUDE B. ELION (by invitation) and HENRY VANDERWERF (by invitation) *The Wellcome Research Laboratories*. The relative effectiveness of 2-aminopurine as an inhibitor of the growth and acid production of *Lactobacillus casei* depends on the nutritives present and the duration of the experiment. This adenine isomer is an active inhibitor when folic acid is present but relatively ineffective when thymine is substituted for folic acid. In a medium containing 2-aminopurine and folic acid, the inhibition of growth can be relieved by the addition of adenine or folic acid. When the period of observation is extended from the usual 72 hours to 13 days the behavior of 2-aminopurine is found to be distinctive. With certain purine antagonists such as 5-amino-7-hydroxy-1-v-triazolo[4,5-d]pyrimidine acid production is continuous with a slight inflection in the curve at about the third day. With 2-aminopurine, however, acid production virtually ceases at the third day though the organisms remain viable. The production of acid by *L. casei* may be regarded as occurring in 2 phases, one of which may be completely inhibited by 2-aminopurine.

**Coupled oxidations in enzymatically oxidized linoleic acid** R. T. HOLMAN (introduced by P. B. PEARSON) *Medicinska Nobelinstitutet, Stockholm and Dept. of Biochemistry and Nutrition, 1 & M College of Texas, College Station*. Using homogeneous crystalline lipoxidase enzyme and linoleic acid substrate at pH 9 the destruction of carotene and bixin added to the system has been studied. It has been found that in this homogeneous lipoxidase assay system used by Theorell, et al., the carotenoid destroyed is proportional to time and to enzyme concentration. Carotenoid oxidized is also proportional to the amount of diene conjugated (peroxide formed). Thus, under these conditions and in the range of concentrations studied, the disappearance of carotenoid is a valid measure of the enzyme activity and can be used as an assay. The oxidative destruction of carotene or bixin in linoleic acid oxidized by lipoxidase is proportional to its own concentration. The amount of diene conjugated decreases as carotenoid concn

tration in the reaction mixtures is increased. It thus appears that the oxidation of carotenoid occurs as the result of the interruption of the chain linoleic acid oxidation reaction by carotenoid molecules. The number of linoleic acid molecules whose conjugation was inhibited by the oxidation of one molecule of carotenoid was found to be approximately 43 for carotene and 26 for bixin.

**The kinetics of inhibitor action in a carrier-linked system.** B. L. HORNECKER and J. N. STANWARD (by invitation). *Laboratory of Physical Biology, National Institute of Health, Bethesda, Maryland.* Warburg and others have shown that the inhibition of respiration by cytochrome oxidase inhibitors increases when substrate is added, and have interpreted these results in terms of the "saturation" concept. Difficulties have arisen in the application of this concept. An alternative explanation is offered based on the kinetics of carrier-linked systems. From a consideration of the steady state, Equation 1 is derived to account for the effect of inhibitor on the overall reaction,

$$(1) \quad \text{Per cent inhibition} = \frac{1 - P}{1 + RP} \times 100$$

where  $R$  is the ratio of oxidase to dehydrogenase activity and  $P$  the fraction of oxidase activity remaining in the presence of inhibitor. The equation was tested by a study of the effect of azide on the succinoxidase system. The activities of cytochrome oxidase and succinic dehydrogenase were measured independently by spectrophotometric observation of the rates of oxidation or reduction of cytochrome  $c$ . The ratio  $R$  was calculated from the first order reaction velocity constants.  $P$  was calculated from the mass law expression for the dissociation of the cytochrome oxidase hydrazoic acid complex, with dissociation constant of  $7 \times 10^{-7}$ . The  $O_2$  uptake of the succinoxidase system was measured manometrically under the same conditions of  $O_2$  tension, temperature and substrate concentration as in the spectrophotometric experiments. The observed inhibition of the  $O_2$  uptake was found to be in agreement with Equation 1. Even at optimal substrate concentrations the overall reaction was inhibited considerably less than the oxidase. The equation accounts for increased inhibition with increase in substrate concentration.

**Studies on cerebroside.** SIF HSIEH GLEN (by invitation), C. E. FARR (by invitation) and HAROLD H. WILLIAMS. *Department of Biochemistry, Cornell University, Ithaca, N. Y.* Previous studies have shown that cerebroside, although prominent constituents of brain and nervous tissues, are present in other organs and tissues, particularly the muscles. Furthermore, they have been shown to have a common structure consisting of the nitrogenous base, sphingosine, the hexose galactose, and fatty acid, variations in the type of

cerebroside being dependent on the particular fatty acid present. Recent work, however, has demonstrated that another type of cerebroside may occur, especially in the spleen in Gaucher's disease. This type is characterized by the presence of a different hexose in the molecule, namely, glucose. In view of these observations, an investigation of the type of cerebroside (with respect to the hexose present) present in muscle and other tissues was undertaken. Cerebroside were isolated from beef and rat brain, beef cardiac muscle, chicken breast muscle and egg. The hexose present was identified by chemical and physical methods and confirmed by specific microbiological fermentation procedures which will be discussed. In all cases, galactose was found to be the only hexose present.

**Phosphorylation of glucose due to a coupled oxidation-reduction between  $\alpha$ -ketoglutaric acid and oxalacetic acid.** F. EDMUND HUNTER, JR. *Department of Pharmacology, Washington University School of Medicine, Saint Louis, Missouri.* Earlier work, *J. Biol. Chem.* 159, 295 (1945), indicated that washed kidney tissue particles catalyzed the following reaction anaerobically:  $\alpha$ -ketoglutarate + oxalacetate  $\rightarrow$  succinate +  $CO_2$  + malate. In attempting to explain the stimulating effect of this reaction on the conversion of acetoacetate to citrate, it was suggested that this anaerobic reaction, like the aerobic oxidation of  $\alpha$ -ketoglutarate, could generate high energy phosphate bonds. The difference between the oxidation-reduction potentials of the oxalacetate-malate system and the  $\alpha$ -ketoglutarate-succinate system is sufficient to permit such a reaction. Investigation reveals that this reaction does result in the creation of high energy phosphate which can be transferred via the adenylate system to glucose under certain conditions. Liver particles are more active than kidney. A homogenate prepared with phosphate Ringer solution is centrifuged at high speed and the precipitate washed three times with phosphate Ringer. When the reaction mixture consists of tissue, oxalacetate,  $\alpha$ -ketoglutarate, phosphate,  $Mg^{++}$ ,  $DPN^+$ ,  $NaF$ , adenylate, and glucose, very little glucose is phosphorylated, but the adenylate is converted to ATP. This is best seen with more than catalytic amounts of adenylate. When purified hexokinase is added with catalytic amounts of adenylate, inorganic phosphate disappears and glucose 6 phosphate is formed. Very little phosphorylation occurs when either oxalacetate or  $\alpha$ -ketoglutarate is omitted. The pyruvate-lactate system, although of similar potential, will not replace oxalacetate-malate. These experiments represent a step toward direct demonstration of the points of origin of the three high energy phosphate bonds which result from aerobic oxidation of  $\alpha$ -ketoglutarate to succinate.

The determination of various amines by proton-exchanges with ethyl eosin in non-aqueous solvents J LOGAN IRVIN and ELINOR MOORE IRVIN (by invitation) *Department of Physiological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore* Various nitrogenous compounds of biochemical interest can be determined in micro-quantities by means of proton-exchanges in non aqueous solvents. For example, 0.1  $\mu$ g of pamaquine can be determined in 10 ml of ethylene dichloride by measurement of the fluorescence of the union of ethyl eosin which results from the transfer of a proton from ethyl eosin (practically non fluorescent) to a proton acceptor group in pamaquine. Of various fluorescein derivatives eosin and ethyl eosin are especially satisfactory because their anions have absorption bands of favorable wavelengths for excitation by the intense 545 m $\mu$  "line" of the mercury emission spectrum. Ethyl eosin is preferable to eosin inasmuch as the esterified carboxyl group of the former does not compete with the desired fluorescence-yielding proton-exchange. In contrast to water, which exerts a leveling effect through its amphoterie property, ethylene dichloride and similar solvents are inert and do not compete with the desired reaction through donation or acceptance of protons. The method has been applied in the determination of various amines such as certain antimalarial compounds, benzedrine, phenylethylamine, and histamine. Application to the latter two compounds suggests use of the method, in conjunction with specific amino acid decarboxylases for micro determination of certain amino acids. Partial separation of primary amines from secondary and tertiary amines can be effected by reaction with a large excess of salicylaldehyde in alkaline aqueous solution. Upon extraction of the solution with ethylene dichloride, the salicylidine-amine, is retained in the alkaline aqueous solution.

Physical dependence liability of drugs of the methadon series and of 6-methyldihydromorphine HARRIS ISBELL (by invitation) and A J EISENMAN *Research Department, U S Public Health Service Hospital, Lexington, Kentucky* Average doses of 21 mgm of racemic methadon (6-dimethylamino-4,4-diphenyl-3-heptanone), 11 mgm of levo-methadon, 90 mgm of dextromethadon, 84 mgm of racemic methadol (6-dimethylamino-4,4-diphenyl-3-heptanone) 78 mgm of racemic isomethadon (5 dimethylamino 4,4-diphenyl-3-heptanone) and 83 mgm of 6-methyldihydromorphine were administered to 10 men, at the 28th to 32nd hour of abstinence from morphine. Levo methadon and racemic methadon reduced the intensity of the abstinence more than did 30 mgm of morphine. Dextromethadon and methadol had no effect. Isomethadon reduced the intensity of abstinence as much as

did 30 mgm of morphine. 6-Methyldihydromorphine had only a small and transient effect.

1 mgm of levo methadon was substituted for each 8-10 mgm of the stabilization dose of morphine in 7 subjects who were addicted to morphine without the appearance of signs of abstinence. Following withdrawal of the levo methadon after 11 days substitution, a definite abstinence syndrome ensued, which was similar to the syndrome seen after withdrawal of racemic methadon. Following the substitution of 1 mgm of isomethadon for each 1.5 mgm of the stabilization dose of morphine, signs of mild abstinence appeared in 2 of 5 cases. When the isomethadon was withdrawn after 11 days substitution, an abstinence syndrome, very similar to the morphine abstinence syndrome, became evident 12 hours after the last dose was administered.

Availability of DL-lanthionine for the promotion of growth when added to a cystine-deficient diet D BRIEF JONES, ARVIN CALDWELL (by invitation), and WILLARD J HORN (by invitation) *From the Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, United States Department of Agriculture, Washington* Lanthionine, the thioether amino acid isolated from various sodium carbonate treated proteins was obtained in two forms, the meso and racemic (Horn, Jones, and Ringel *J Biol Chem*, 111, 87 [1912] 114, 93 [1912]). It was later shown that the internally compensated mesolanthionine can not serve in lieu of cystine in the diet of the rat (Jones, Divine, and Horn *J Biol Chem*, 116, 571 [1912]). Inasmuch as rats are unable to utilize D cystine for growth, their failure to grow on a cystine deficient diet supplemented with mesolanthionine indicates that they are unable to cleave the thioether amino acid or that cleavage occurs with the formation of the unutilizable D cysteine instead of L cysteine. It therefore seemed possible that Lanthionine can be utilized for growth. Feeding experiments have now shown that racemic lanthionine can replace cystine and methionine in the diet. Young albino rats fed a low protein (casein) basal diet deficient in cystine but adequately supplied with the non-protein dietary essential factors declined in weight rapidly. For comparison with the behavior of rats given racemic lanthionine, L cystine and DL methionine were also used. Addition of 0.3 gm of L-cystine or 0.37 gm of DL-methionine per 100 gm of basal diet caused immediate resumption of growth. Addition of DL-lanthionine likewise resulted in corresponding growth resumption. An immediate decline occurred when the lanthionine was omitted from the basal diet, and growth again was resumed when the lanthionine was added.

Citrate formation from oxalacetate GEORGE KALNITSKY (introduced by H A MITCHELL)

*Department of Biochemistry, State University of Iowa, Iowa City* Rabbit kidney cortex homogenates form citrate from oxalacetate. Phosphate and bicarbonate- $\text{CO}_2$  buffers were most suitable. Borate and veronal buffers inhibited at higher (0.04M) concentrations. Addition of inorganic phosphate to homogenates resulted in slightly increased citrate formation. This was not due to buffering effect of the phosphate. Active cell free preparations were obtained by centrifuging homogenates (1:2 dilution) for 4-5 minutes at approximately 3000 rpm.  $Q_{\text{citrate formation}}$  was 6.9. The optimum pH was 7.3-7.9 with the peak at 7.5-7.6. Optimum substrate concentration was 0.02M (100  $\mu\text{M}$ ). Formation of citrate was proportional to enzyme concentration up to 1.5 ml of enzyme solution in a total volume of 5 ml. Addition of pyruvate or acetoacetate did not appreciably increase citrate formation. An atmosphere of air or  $\text{O}_2$  was equally effective; anaerobically, under  $\text{N}_2$ , 50% less citrate was formed. Dialysis for 15 to 20 minutes resulted in a marked decrease (66%) in citrate formation. Most of this activity was restored on addition of inorganic phosphate. The necessity of inorganic phosphate for citrate formation from oxalacetate might be interpreted as evidence for (1) the formation of a phosphorylated intermediate, possibly phosphocitrate, or (2) the combination of oxalacetate with a two carbon compound, according to the following reactions:

$$\begin{aligned} &\text{oxalacetate} \xrightarrow{\text{PO}_4} \text{pyruvate} + 2 \text{ c compound} \\ &\text{oxalacetate} + 2 \text{ c compound} \rightarrow \text{citrate} \end{aligned}$$

After dialysis for 50 minutes against distilled water the preparation was inactivated and could not be reactivated by addition of inorganic phosphate, boiled kidney juice and adenosinetriphosphate.

**Reactions between acetate, acetyl phosphate and the adenylic acid system in tissue and bacterial extracts.** NATHAN O. KAPLAN (by invitation) and FRITZ LIPMAN, *Biochemical Research Lab., Massachusetts General Hospital, and the Department of Biological Chemistry, Harvard Medical School, Boston*. Phosphate exchange between acetyl phosphate and the adenylic acid system had readily been observed with various bacterial extracts, either by direct reaction or by using glucose as the ultimate P acceptor. A transfer of acetyl P to glucose has now been observed also in pigeon liver extracts. In aged acetone powder extracts, a fixation of from 15 to 55% of the added acetyl P occurred, using fluoride and citrate as inhibitors of interfering enzymes. Higher values of 36-55% were obtained with added ADP. On omitting glucose, no fixation took place. This phosphate transfer from acetyl phosphate to the adenylic acid system does not require coenzyme A, although Co A is necessary for acetyl transfer from acetate plus ATP. Recently, in studies with *E. coli* extracts acetate

plus ATP were found to yield large amounts of acetyl phosphate without addition of an acetate acceptor. The compound formed seems to differ, however, from synthetic monoacetyl phosphate by being quite resistant to the action of an earlier described specific muscle acetyl phosphatase. Efforts are underway to test for the existence of two acetyl-P derivatives by isolation of the product of the reaction between ATP and acetate.

**Isolation and assay of an anti-secretory substance from duodenal mucosa.** J. KATZ (by invitation), ROBERT L. DRYER (by invitation), W. D. PAUL (by invitation), and J. I. ROUTH, *Department of Biochemistry and Internal Medicine, College of Medicine, State University of Iowa, Iowa City*. Extracts have been prepared from lyophilized de-fatted duodenal mucosa of hogs according to the method of Kim and Lim. Various methods have been applied to concentrate and purify a substance from these extracts which actively inhibits gastric secretion and gastric ulceration. The concentrates were tested by intraperitoneal injection of 1 cc into rats with ligated pylorus. The animals were prepared by fasting 72 hrs with free access to water. Pyloric ligation was performed under light ether anesthesia, and the extracts were injected immediately following ligation. After 6 hr the stomachs were excised and the volume of the contents was measured and compared with that from control rats, with ligated pylorus, injected with 1 cc of normal saline. Free acidity, total acidity, pH, and peptic activity was determined in all samples. The interior of each stomach was examined with a dissecting microscope. Our results indicate that the material isolated from these extracts possesses an inhibitory effect on gastric secretion, an effect which we are attempting to utilize as an assay method. The anti-ulcer activity of this substance is also under study. Attempts are being made to correlate the anti-secretory and anti-ulcer effects with the physical and chemical properties of the active agent.

**Application of the isotopic derivative method of analysis to protein hydrolysates.** ALBERT S. KESTON (by invitation), SIDNEY UDENFRIEND (by invitation), and R. KEITH CANNAN, *Department of Chemistry, New York University College of Medicine, New York*. The isotopic derivative method, recently reported (Keston, Udenfriend, and Cannan, *J. A. C. S.*, 68, 1390 (1946)), has been modified and further applied. Various techniques for separating the homologous derivatives were studied to separate different carriers as well as to aid in their purification. Distribution coefficients of many p-tolophenyl sulfonfyl (pipsyl) amino acids between various solvent pairs were determined. The relative distribution coefficients of the pipsyl amino acids between chloroform and water are

almost the same as those of the acetyl derivatives (Syngé, *Biochem J* 33, 1913 (1939)) although the two substituent groups differ greatly in size. The distribution coefficients were used to develop countercurrent distribution procedures for the separation of the different derivatives, making it possible to add several carriers to a protein hydrolysate which has been treated with isotopic pipsylchloride, and to obtain analyses for several amino acids on a single aliquot. By using several different techniques for purification of individual carriers purity is more readily achieved. Countercurrent solvent extractions, recrystallizations, and differential adsorption on norite were all used to achieve purification of the isolated carriers. Recoveries of glycine, alanine, and proline from known mixtures of amino acids have been highly satisfactory. When the amino acid being estimated was omitted from the mixture none was found by the method. This demonstrated the separability of homologous isotopic impurities from the carriers. Analytical values have been obtained for glycine, alanine, and proline in  $\beta$  haetoglobulin, human hemoglobin, rabbit aldolase, and rabbit phosphoglyceraldehyde dehydrogenase, using samples of protein of the order of 1 mg.

**Quantitative analysis of protein hydrolysates on paper chromatograms by means of the isotopic derivative method.** ALBERT S. KESTON (by invitation), SIDNEY UDENFRIEND (by invitation), and MILTON LLEVY. *Department of Chemistry, New York University College of Medicine, New York.* The isotopic derivatives obtained in the method of analysis described by Keston, Udenfriend, and Cannan (*JACS*, 68, 1390 (1946)) are suitably separated by paper chromatography. The combination offers a method applicable to the estimation of amino acids in microgram quantities of proteins. A sample of derivatives, labelled with  $p\text{-I}^{131}\text{-C}_6\text{H}_4\text{SO}_2\text{Cl}$  ( $\text{I}^{131}$  pipsylchloride) is placed as a transverse line on a paper strip and the chromatogram developed with n-pentanol saturated with 2 N  $\text{NH}_3$ . Different derivatives are located as radioactive bands with a G.M. counter or radioautography, and are initially identified from retardation factors. The counts in a resolved band divided by  $C_r$ , counts per mole of substance made with the same reagent, equals the moles of substance. Identity and purity of a band is established by eluting, adding corresponding non isotopic derivative as carrier, and demonstrating unchanged isotope concentration on purification. Estimations may be made independent of complete resolution of bands by adding indicators (either unlabelled derivative or derivative labelled with a second isotope) before chromatography. Estimations then depend on isotope ratios in any pure sample. To determine an amino acid, its  $\text{S}^{35}$  labelled pipsyl derivative (B counts) is added as indicator to the

derivatives obtained from a hydrolysate and  $\text{I}^{131}$ -pipsylchloride. Where the ratio of  $\text{I}^{131}/\text{S}^{35}$ , (R), in successive strips is constant it can be used to calculate the amount of amino acid originally present, moles of amino acid =  $\text{RB}/\text{C}$ . R is readily calculated from measurements made with and without an Al filter.

**A new adenosinetriphosphatase of muscle.** W. WAYNE KIRKLEY (introduced by OTTO MEYERHOF). *Department of Physiological Chemistry, School of Medicine, University of Pennsylvania.* A highly unstable ATPase has been isolated from muscle by extraction with dilute alkaline salt solution, repeated fractionation with 0.3 to 0.4 saturated  $(\text{NH}_4)_2\text{SO}_4$  and high speed centrifugation (18000 r.p.m.). This enzyme which splits ATP to ADP is activated by Mg, inhibited by Ca, and has an optimum activity around pH 7.4. In all these respects it differs from the myosin ATPase. It is present in fresh muscle in about the same amount as the myosin ATPase and can be brought to the same degree of absolute activity (purity) as the latter. In the presence of some ampholytes the activation and pH optimum of myosin may be shifted toward the properties of this new preparation. The relation between the two enzymes will be discussed.

**Effect of carbon dioxide concentration on the changes in brain metabolites accompanying convulsions.** J. RAYMOND KLEIN and JAMES A. BIRN (by invitation). *Depts. of Psychiatry, Biological Chemistry, and Pharmacology, University of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago.* Convulsions in rats breathing a mixture containing 15% carbon dioxide and 85% oxygen were found to be accompanied by an increase in brain lactate, a decrease in glucose, and no obvious decrease in concentration of adenosine polyphosphates or phosphocreatine. The absolute increase in brain lactate was less than that found in convulsed animals breathing room air and the level attained was about the same as in unconvulsed animals breathing room air. The lack of change in labile phosphates in the convulsed animals breathing the carbon dioxide mixture contrasts with the considerable decreases occurring in convulsed animals breathing room air.

**Selenium tetraglutathione.** HARLAN L. KLEGG (by invitation), GEORGE P. LAMPSON (by invitation) and ALVIN L. MOXON. *Chemistry Department, South Dakota Agricultural Experiment Station, South Dakota State College, Brookings, South Dakota.* Sodium selenite (subcutaneously injected) has been shown to decrease liver and blood reduced glutathione (GSH) values in rats in this laboratory. These results suggested that selenite had combined with GSH in the tissues, therefore an investigation of the reaction between selenite and GSH was initiated. The compound was prepared by mixing

aqueous solutions of 0.1 mole GSH and 0.025 mole selenous acid, ethanol was added until a white precipitate formed, the product was cooled overnight on ice, filtered, washed with ethanol, and dried in a vacuum desiccator. Analysis showed a sulfur to selenium ratio of 4:1. The crystalline compound is white, slightly soluble in cold  $H_2O$ , moderately soluble in hot  $H_2O$ , and is stable in an acid solution. Decomposition occurs in an alkaline solution with liberation of elemental selenium, and also upon heating the compound to  $140^\circ C$ .

Tests on an aqueous solution show neither free SH groups nor excess selenous acid, but reduction by zinc dust in acid solution liberates elemental selenium and SH groups which can be recovered in theoretical amounts when titrated with  $KIO_3$ . Tests show only a trace of disulfide sulfur, which indicates that practically all the sulphur is bound to selenium. These tests indicate further that the product formed is a selenium tetraglutathione with one Se atom bound to the four S atoms. The compound is significant in selenium toxicity studies as it may assist in explaining the detoxication effect of SH groups on selenium. Toxicity studies show that the selenium in this compound is markedly less toxic than selenium in sodium selenite, which indicates that the combining of selenite with GSH converts the selenium to a less toxic form.

**Studies on the micro molecular distillation of blood lipids.** ALFRED C. KOEHLER, ELSIE HILL (by invitation) and FRANK FEARNEY (by invitation). *Santa Barbara Cottage Hospital and The Sansum Clinic Research Foundation, Santa Barbara, California.* Further studies of the distillation of serum lipids from a film at 0.03 micron Hg at comparatively low temperatures were made. It was found that  $58^\circ C$  was the most suitable temperature for the separation of cholesterol from its esters. This separation agreed closely with the digitonin method in normal sera but when the esters were low the free cholesterol was about 10% lower than that by the digitonin separation. This indicates the presence of a substance that gives the Liebermann-Burchard color which is precipitated by digitonin but is not distilled under conditions that completely distill free cholesterol. Fatty acids can readily be distilled, partially even at room temperature, 50% at  $35^\circ C$ , 90% at  $128^\circ C$ . After phospholipid and free cholesterol separation by precipitation, the fatty acid glycerides can be partly separated from the cholesterol esters by distillation at  $100^\circ C$  or lower inasmuch as no natural cholesterol esters distill below  $100^\circ C$ . Approximately 50% of the blood fats distill at  $55^\circ C$ . Fractionation of the combined fatty acids of the blood filtrate, as well as the fats shows a fairly uniform composition of different sera in health and a variety of disease conditions. Some

notable exceptions were found however, especially in some instances of diabetic ketosis.

**DPN pyrophosphatase.** ARTHUR KORBERG and OLOF LINDBERG (introduced by CARL F. CORI). *Department of Biological Chemistry, Washington University School of Medicine, St. Louis.* Enzymes which split diphosphopyridine nucleotide (DPN) are found in microorganisms, in plant and in animal tissues. The mode of action of these enzymes is not well understood. Handler and Klein (J. Biol. Chem. 143:49, 1942) reported that in animal tissues the nicotinamide ribose linkage is the primary and principal site of cleavage. Heuvelink (Arkiv Kemi, Mineral o Geol. 13A:19, 1939) observed the formation of adenylic acid when DPN was incubated with sweet almond press juice. We have observed that washed rabbit kidney particles convert added DPN to adenylic acid and a product which has been identified as nicotinamide ribose phosphate. In the presence of an oxidizable substrate (i.e. glutamate) as a source of phosphate bond energy, adenosine triphosphate is formed in place of adenylic acid. These and other observations indicate that the pyrophosphate bond is the primary site of DPN cleavage in these preparations.

DPN is split more rapidly in a respiring system (i.e. with added glutamate) than in a non-respiring one. This fact may be explained by the more rapid rate of splitting of the reduced form of DPN as compared with the oxidized form, as measured under anaerobic conditions.

**The determination of the biological indices of gluten and gluten fortified with lysine in normal and protein-deficient patients.** DONALD D. KOZOLL (by invitation), WILLIAM S. HOFFMAN, GORDON McNEIL (by invitation) and HANS POPPER (by invitation). *From the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois.* The method of Allison was applied to 10 human subjects in whom gluten (G) and gluten fortified with 4% lysine (GL) were administered orally. Seven patients were protein deficient, 3 normals were studied for comparison. All patients were kept 3 to 9 days on a protein free diet for the determination of the endogenous nitrogen excretion. Thereafter, 3 protein deficient subjects were fed for 5 day periods progressively increasing levels of intake of G and GL. In the remaining 7 subjects a simplified procedure was utilized consisting of 10 day periods during which equivalent quantities of G or GL calculated to barely approach equilibrium were given. In 3 subjects an additional 10 day period was employed wherein the patient returned to G.

The biological index (K) for G varied from 0.70 to 1.03, for GL this value varied between 0.52 and 1.22. The calculated quantities of nitrogen required for equilibrium for G varied between .49 and 1.09

mg per kilo per day, whereas, for *GL* it ranged between 39 and 98 mg per kilo per day. No differences were discernible between normal and protein deficient subjects. Where patients were returned to *G*, results were similar to those initially obtained with *G*. Light subjects were submitted for statistical analysis. The average *K* value for *G* was 0.63 and for *GL* was 0.78. The average nitrogen requirement for nitrogen equilibrium for *G* was 83 mg per kilo per day and for *GL* 68 mg per kilo per day. The conclusions are drawn that the determination of the biological index of Allison is applicable to the study of an oral protein in the human protein deficient subject. Although gluten proved to be a fairly complete protein, gluten fortified with 1% lysine had a higher biological value.

**Immunochemical studies on purified d-glyceraldehyde-3-phosphate dehydrogenase from yeast and rabbit muscle.** LOWIN G. KRIBBS (by invitation) and VICTOR A. NASSAR, *Department of Biological Chemistry, Washington University School of Medicine*. Antibodies for d-glyceraldehyde-3-phosphate dehydrogenase from yeast have been produced in rabbits. The enzyme used for the antigen was obtained from baker's yeast by a simplification of the procedure reported by Warburg and Christian and was 85% pure by electrophoretic measurement. Several recrystallizations led to electrophoretically homogeneous enzymes, but this was not used for immunization. Rabbits were given a series of subcutaneous injections of the antigen and developed precipitating antibodies. Precipitin tests with undiluted serum were positive to a 1:10,000 dilution of the 4 per cent antigen solution. The activity of the yeast enzyme was inhibited 80 per cent by a 1:30 dilution of the antiserum in the reaction mixture. No inhibition of crystalline muscle d-glyceraldehyde-3-phosphate dehydrogenase was noted under the same conditions. Further studies are being undertaken to determine whether antibodies to the yeast enzyme produced in a different animal will inhibit the rabbit enzyme.

The crystalline yeast and rabbit enzyme were compared in other properties. Kinetically the specific activities are of the same magnitude, and both enzymes are activated by cysteine. This latter fact was not reported by Warburg for the yeast enzyme. Taylor et al. (in press) have found that the muscle enzyme crystallizes with DPN as an essential component of the crystalline structure. No DPN has been detected in the crystalline yeast enzyme. At pH 7.3 the electrophoretic mobility of the yeast enzyme is much greater than that of the muscle enzyme.

**Nutritional studies on the cat.** W. A. KREHL and I. D. WELT (by invitation), *Yale Nutrition Laboratory, Department of Physiological Chem-*

*istry, Yale University*. No adequate data are available concerning the nutritional requirements of the cat, an animal which may be profitably used for nutritional investigations. The present communication represents a successful attempt to maintain the growth of kittens to maturity upon a completely "synthetic" diet. In our experience cats would not eat the usual synthetic rat diet. A diet modeled after the composition of solids in cow's milk resulted in maintenance, but was inadequate for growth. A ration based upon the composition of the solids of bitch's milk, an animal with a rapid growth rate similar to that of the cat, was then devised. All recognized vitamins of the B complex, as well as synthetic vitamin K, were added to this basal ration. A concentrate containing appropriate amounts of vitamins A, D, and E was administered weekly by mouth.

Three female litter-mate kittens, one litter-mate male, and one non litter-mate male were placed upon this ration. It has proved to be eminently satisfactory for growth beyond maturity to a degree comparable to that reported in the literature for kittens fed the usual "house" diet. The adequacy of this diet for normal growth was confirmed as follows. One male and two female kittens were placed on a similar diet, to which 1% of whole liver powder was added at the expense of lard. No significant difference could be detected between the growth rates of the two groups of animals. Blood studies were also carried out and will be reported.

**The availability of amino acids in some foods.** R. A. KLUICKEN (by invitation) and CARL M. LEMAN, *Department of Biochemistry and Nutrition, A and M College of Texas, College Station*. The extent to which the individual amino acids in a number of foodstuffs are available for use by the rat was studied by determining the 10 essential amino acids in the food and in the feces. Young rats were fed rations in which practically all of the protein was supplied (at a 10% level) by a single foodstuff. The experimental period was followed by a control period in which a ration containing 4% whole egg protein was fed. The data obtained from the control period were used to correct the apparent availabilities for the amino acids normally present in the feces as a result of metabolic processes and bacterial action.

The results showed that the individual amino acids in a given foodstuff are not always equally available. For example, the value obtained for the availability of arginine in cottonseed flour was 93.4% while the value for the availability of lysine in the same sample was 64.5%. In the case of meat all 10 amino acids were 100% available. The corresponding values for wheat ranged from 92.2 to 98.5% and for peanut flour from 94.8 to 99.5%.

**The fatty acid oxidase complex of rat liver and**



intracellular structures ALBERT L LEHNINGER and EUGENE P KENNEDY (by invitation) *Departments of Surgery and Biochemistry, University of Chicago, Chicago* In a recent investigation (Lehninger and Kennedy, *J Biol Chem*, submitted) the requirements for activity of the fatty acid oxidase system have been more completely defined Saline washed particulate matter of rat liver after treatment with water requires the presence of adenine nucleotide,  $Mg^{++}$ , inorganic phosphate, neutral salts such as KCl (or certain non electrolytes such as sucrose), cytochrome c and catalytic amounts of l malate or oxalacetate for restoration of ability to oxidize octanoate to acetoacetate The function of the neutral salt is to cause transformation of the enzyme complex from an inactive "dispersed" condition to the enzymatically active, "flocculated" state This behavior of the enzyme complex suggested possible identity with the "mitochondria" or "large granules", separable from liver extracts by differential centrifugation, which are known to "agglutinate" in isotonic saline Also, the highly organized nature of the enzyme complex, which also catalyzes the reactions of the Krebs cycle and coupled phosphorylations, suggested that the particles preexist as such in the cell Fractionation of the particulate matter of rat liver in 30% sucrose (Hogeboom, Schneider and Pallade, *Proc Soc Exptl Biol Med*, 65, 320 (1947)) revealed that the washed fraction designated by Hogeboom et al as consisting of morphologically intact mitochondria free of extraneous elements, contains essentially all of the fatty acid oxidase activity of rat liver as well as Krebs cycle activity

Enzymatic oxidation of tyrosine and dihydroxyphenylalanine by melanoma extracts ABRAHAM BLISSER LERNER (by invitation) THOMAS B FITZPATRICK (by invitation), EVAN CALKINS (by invitation), and WILLIAM H SUMMERSON *Biochemistry Section, Medical Division, Army Chemical Center, Maryland* The enzymes tyrosinase and dopa oxidase in melanoma tissue have been considered to be separate substances catalyzing the oxidation of tyrosine and dihydroxyphenylalanine (dopa) respectively Experiments to be described indicate that the enzymatic oxidation of both of these substances by active fractions from mouse melanoma tissue can be accounted for in terms of a single enzyme This enzyme appears to be actually a tyrosinase, with dopa, or a product of dopa oxidation, serving merely to catalyze the reaction in which tyrosine is converted to melanin

Our evidence indicates that this enzyme, like plant tyrosinase, is a copper protein Analysis of enzyme preparations for iron, cobalt, magnesium, manganese and copper revealed that only copper is present in significant amounts When substances which combine with copper, e.g., sodium diethyl

dithiocarbamate, phenylthiourea and others were added to enzyme preparations, the tyrosinase activity was inhibited Enzymatic activity was restored by the addition of an excess of copper Other metals did not produce this effect

The effect of testosterone and methyl testosterone on guanidoacetic acid in blood and urine BLAINE H LEVEDAHL (by invitation) and LEO T SAMUELS *From the Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah* With 3 male subjects maintained on a dose of 50 mg of methyl testosterone per day, the blood and urine levels of creatine and guanidoacetic acid were found to increase sharply after an initial delay of approximately 30 days Ten days after the cessation of methyl testosterone administration the creatine and guanidoacetic acid levels of both the blood and urine were found to be normal Significant changes in the blood and urine creatinine levels were not observed during the test period With 3 female subjects given 50 mg of methyl testosterone per day, the rapid use in the creatine and guanidoacetic acid levels was found to occur approximately 12 days after the start of administration Increases of 3 to 6 times the normal urine creatine and guanidoacetic acid levels as well as increases of 2 times the normal blood levels of creatine were observed in a period of 10 days No significant changes in the blood and urine creatine levels were noted

Testosterone propionate given by injection, implantation or oral administration to four male subjects for 40 days showed only a decrease in the urine creatine level after approximately 25 days of administration Methyl testosterone appears to increase formation of guanidoacetic acid as well as creatine and therefore is probably not primarily involved in transmethylation

The effect of gonadotrophic stimulation on the cholesterol content of the immature rat ovary LOUIS LEVIN and JOSEPH W JAILER (by invitation) *Departments of Anatomy and of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York* Bloch has shown that fed cholesterol is excreted in part as pregnandiol, the conversion presumably being by way of progesterone It may be expected that during the ovarian secretory process, ovarian cholesterol is utilized for conversion to estrogen and/or progesterone Accordingly, a comparison has been made of the cholesterol content of the immature rat ovary before and after treatment with pituitary gonadotrophin Injection of this gonadotrophin causes a rapid loss of ovarian cholesterol (as much as 20% loss) followed by a gradual return toward the original level and a final attainment of levels far above the original The loss of ovarian cholesterol is evident within 1 hour after a single injection of gonadotrophin and is maximal at 3 to 12

hours. Thereafter, cholesterol replenishment begins, the original level being nearly attained at 24 hours and being surpassed (double the original level) at 48 hours. The cholesterol accumulation continues at least until 100 hours after gonadotrophin administration. Due to the loss of cholesterol the concentration in the ovaries initially drops sharply. Later, during the phase of cholesterol accumulation, the concentration does not show any substantial increase because of the coincident increase in ovarian weight.

Human chorionic gonadotrophin, in adequate dosage, causes a loss of ovarian cholesterol similar to that caused by pituitary gonadotrophin. Such loss does not occur after injection of pituitary adrenotrophic hormone nor after exposure of the animal to cold, but these treatments do cause a loss of adrenal cholesterol.

**Dibenzothiophene as a reagent for aldehydes, ketones and carbohydrates.** VICTOR L. LEVINE and SIDNEY MERLIS (by invitation), *Department of Biological Chemistry and Nutrition, Creighton University School of Medicine, Omaha, Nebraska*. Dibenzothiophene condenses with carbohydrates and certain aldehydes and ketones in the presence of concentrated sulphuric acid with the formation of colored complexes. The reagent is 1% dibenzothiophene in alcohol. If the tested substance is in aqueous solution, the reagent is likely to precipitate. This precipitation does not interfere with the formation of a ring or zone of characteristic color, when reagent and solution are underlaid with sulphuric acid. Pentoses and pentose yielding compounds (arabinose, xylose, ribose, fucose, rhamnose, gums, nucleic acid) yield a deep red ring. Hexoses and hexose yielding compounds (glucose, fructose, sorbose, galactose, mannose, cellose, sucrose, maltose, melibiose, lactose, melezitose, raffinose, dextrin, starch, inulin, egg and serum albumin, casein, amygdalin, esculin) yield a blue ring changing to purple on shaking. A number of aldehydes and ketones form a pink ring. Acetaldehyde reacts, but acetone does not. p-Dimethylaminobenzaldehyde forms a purple ring. Formaldehyde yields an intense green color, specific and very sensitive, one gamma per milliliter being readily detectable. Formic acid does not interfere. Paraformaldehyde also reacts, as well as compounds which are condensation products of formaldehyde with ammonia (methenamine, methenamine mandelate) and with alcohols or thio alcohols (methylal, ethylal, propylal, djenkolic acid). The benzothiophene reaction detects formaldehyde in milk and also methyl alcohol after oxidation. It also serves to identify methyl alcohol in ethyl alcohol. Oxidation produces formaldehyde and acetaldehyde. The reagent in the presence of these two aldehydes forms an

upper pink or reddish ring with acetaldehyde and a lower deep ring with formaldehyde.

**The arrangement of amino acids in silk,** an application of the isotopic derivative technique. MIRIAM LEVY and IRENE SLOBODANSKY (by invitation), *Department of Chemistry, New York University College of Medicine, New York*. Using  $p$ - $^{14}\text{C}_6\text{H}_5\text{SOCl}$  (pipsyl chloride) and the principle described by Keeton, Udenfriend, and Cannan (*J. A. C. S.*, **68**, 1390 (1946)), hydrolysates of silk fibroin, containing dipeptides, have been analysed for alanine, glycine,  $\alpha$ -amylglycine, glycylalanine, and glycylglycine. The hydrolysates were prepared by the action of 12 N HCl at 39° for 16 to 48 hours. The 48 hour hydrolysate contained, in per cent of N, 12% of glycine N, 9.5% of alanine N, 1.5% of glycylglycine N, 8% of glycylalanine N, and 2.5% of  $\alpha$ -amylglycine N. All the values were lower at 24 hours except glycylalanine which was unchanged. Total hydrolysis of the same silk fibroin gave 39% glycine N and 26% alanine N. Practically all of the alanine and about half of the glycine are accounted for in the 48 hour hydrolysate. The results are incompatible with a random arrangement of the amino acid residues in the protein.

**The excretion of labeled calcium by normal and thyroparathyroidectomized rats.** MAURICE V. L'HERGUE (by invitation), WILBUR R. TWIFEDY, and LILIAN M. ZORN (by invitation), *Department of Chemistry, Loyola University School of Medicine, Chicago*. Young adult rats which were maintained on a stock diet containing 1.18% of calcium and 0.86% of phosphorus were used. Following the subcutaneous administration of 7 to 14 mgm. of labeled calcium (as  $\text{CaCl}_2$ ) to 8 normal rats (av. wt. 217 gms.), 2.9% of the injected radio calcium appeared in the urine within 6 hours. At the end of 24 hours, 4.1% of the administered radio calcium was detected in the urine, and 8.9% in the feces and intestinal contents. In 2 other normal rats (av. wt. 240 gms.) the excretion of injected radio calcium was determined daily for 8 days. At the end of this period 8.6% of the radio calcium had been excreted in the urine and 18% in the feces.

Eight rats were thyroparathyroidectomized, and 1 to 39 days later injected with labeled calcium and sacrificed after 24 hours. 4 animals which showed terminal serum calcium values of 5.6 to 7.9 mgm. %, excreted 50 to 70% of the amount of radio calcium excreted by their controls. The other 4 animals, whose terminal serum calcium values ranged from 8.3 to 10.4 mgm. %, excreted 101 to 117% of the amount of radio calcium excreted by their controls.

**Adrenal cortical metabolites in human urine.** SEYMOUR LIBERMAN (by invitation), DAVID K. FUKUSHIMA (by invitation), and KONRAD DOBRINER, *Sloan-Kettering Institute for Cancer Research*. Four new metabolites of adrenal cortical

steroids have been isolated from human urine. They are (I) pregnanol  $3\alpha$  dione-11,20, m p  $174.5-5^\circ$ ,  $[\alpha]_D^{25} = +111^\circ$ , (II) androstanol  $3\alpha$  dione-11,17, m p  $175-175.5^\circ$ , (III) etiocholane- $3\alpha,11\beta$ -one-17, m p  $235-7^\circ$ , and (IV) pregnane- $3\alpha,20\alpha$ -one-11 (isolated as the diacetate, m p  $231.5-3^\circ$ ,  $[\alpha]_D^{25} = +56.7^\circ$ ). These were identified by infrared spectroscopy. In each instance the structure was confirmed by comparison with authentic samples prepared by independent methods. (I) and (IV) upon oxidation with  $\text{CrO}_3$  yielded pregnanetrione  $3,11,20$  identical with the known compound. The presence of the  $\text{C}_{11}$ -oxygen function in these compounds makes it almost certain that they are of adrenal origin. Since two of the products are  $\text{C}_{19}$  steroids, they can be presumed to arise from precursors such as corticosterone, the antecedents of the two  $\text{C}_{19}$  compounds cannot be so readily assigned.

There have been isolated from urine by us and others eight ketosteroids of unquestionable adrenal origin. In addition to the four reported above, pregnane- $3\alpha,17\alpha$ -one-20, androstanediol- $3\alpha,11\beta$ -one-17, etiocholane- $3\alpha$  dione-11,17, and  $\Delta^5$  pregnane- $3\beta,17\alpha$ -one-20 have also been isolated. Three transformation products related to these adrenal metabolites have been described. They are  $\Delta^3$  androstanol  $3\alpha$ -one-17,  $\Delta^3$  etiocholane- $3\alpha$ -one-17 and  $17\alpha$  methyl  $\Delta^3$ -D-homoandrostanediol- $3\beta,17\alpha$  ( $\alpha$ )-one-17. The significance of these results for normal and abnormal steroid metabolism in man will be dealt with in the discussion.

**New penicillin products for prolonged blood levels.** LEO LOEWE (by invitation), ALBERT SOBEL, OSCAR GILSON (by invitation) and ERNA ALTMAN WILBER (by invitation). *Departments of Medicine and Biochemistry, The Jewish Hospital of Brooklyn 18, New York.* A study was made of various preparations of penicillin in order to obtain sustained blood levels with a single subcutaneous or intramuscular injection. The following products gave levels for a period of 24 hours or more in rabbits. With similar amounts of penicillin (as the sodium salt) in aqueous solutions no measurable levels were obtained after 6 hours, by the method employed [J. Biol. 18, 599, (1944)].

- 1) Gelatin and penicillin salt mixtures treated with formaldehyde
- 2) Penicillin salts dissolved or suspended in polyoxyalkylene derivative of sorbitan mono-laurate
- 3) The precipitate obtained by the treatment of (serum or milk) protein penicillin mixture with the salts of metal ions which form insoluble derivatives with both protein and penicillin. The metallic ions chosen were those which are nutritionally required (Fe, Cu,

4) Products of type 1) and 3) suspended in polyoxyalkylene derivative of sorbitan mono-laurate

**On the origin of stercobilin in humans.** IRVING M. LONDON (by invitation), RANDOLPH WEST, DAVID SHEWEN and D. RITTEBERG. *From the Departments of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital in the City of New York.* The feeding to humans of glycine labelled with  $\text{N}^{15}$  results in the incorporation of  $\text{N}^{15}$  in the heme of the red blood cells during their formation in the bone marrow. The labelled porphyrin remains in the red cell until the cell disintegrates. The destruction of the cells begins about 70 days after the formation of the cells and reaches a maximum at 128 days. The porphyrin is not reutilized for new hemoglobin synthesis but is excreted in large part as stercobilin. We have isolated crystalline stercobilin from the stools of subjects fed  $\text{N}^{15}$  labelled glycine for two days. The data indicate that not all the stercobilin results from the destruction of hemoglobin. For example, in one experiment the stercobilin isolated from the stools of the first eight days of the experiment contained 0.480 atom % excess  $\text{N}^{15}$ . During this period the cells being destroyed were formed before the administration of labelled glycine and therefore contained no labelled heme. Consequently one can conclude that a significant portion of normal stercobilin production is derived from sources other than hemoglobin. From a consideration of the  $\text{N}^{15}$  concentrations in the stercobilin and heme, it would appear that there is a source of stercobilin other than the known porphyrin protein systems. Further work is in progress to delineate more clearly the relationship between hemoglobin destruction and stercobilin formation and to determine the other biological sources of bile pigment.

**Fractionation of liver proteins.** J. MURRAY LUCK and A. CLARK GRIFFIN (by invitation). *Department of Chemistry, Stanford University, California.* A study has been made of the liver proteins of rats on standard diets with preliminary fractionation studies carried out on the livers of dogs and rabbits. The animals, anesthetized with nembutal, were perfused with Ringer-Locke solution until the livers were virtually blood free. Fractionation of the proteins was then effected by successive extractions of the homogenized liver at pH 7 to 7.5 with sodium chloride solutions of varying molarity. The following fractions were obtained: albumin, globulin, ribonucleoprotein, desoxyribonucleoprotein, and final residue. Extraction of the ribonucleoprotein assumed to be derived from the cytoplasm could not be achieved by the use of 0.14 M sodium chloride (physiological saline). The studies, quantitative in character, include the livers of normal rats, livers in the pre-

cancerous state, and livers in which pronounced tumors had appeared. Azo dyes were used as the carcinogenic agents. All fractionations were carried out at 1°C. The values found for total liver ribose and total desoxyribose in the normal rat approximate those reported in the literature.

**Studies on the agglutination of human and rat red cells by castor bean extracts.** STEPHAN LUDWIG and ALFRED CHANTIN, *Biochemical Laboratory, University of Virginia*. It is well known that castor bean extracts will agglutinate red, white and tissue cells. An attempt has been made to determine the influence of a variety of physical and chemical changes which affect agglutination. Data will be given for human and rat red blood cells. Since rat cells are comparatively fragile it was necessary to determine conditions which would prevent hemolysis and this was done by adding small amounts of serum albumin. It was found that plasma, serum albumin, globulins, casein and other proteins affect the degree of agglutination. In addition, ionic strength, different halogens and pH influence this phenomenon. The red cells of rats poisoned with castor bean extracts behave similarly to those of control animals. The technique for the quantitative determinations of agglutination is critical and the procedures will be given.

**Effect of vitamin B<sub>6</sub> on the utilization of D amino acids by lactic acid bacteria.** CARL M. LYMAN and K. A. KUIKEN (by invitation), *Department of Biochemistry and Nutrition, A and M College of Texas, College Station*. It was found that the ability of *Lactobacillus arabinosus* to utilize the non natural forms of the amino acids was radically altered when pyridoxamine was substituted for pyridoxine in the basal medium. For example, only negligible amounts of the D forms of isoleucine and leucine were utilized when the medium contained pyridoxine, but considerable amounts of these isomers were utilized when the medium contained pyridoxamine. The extent of utilization of D isoleucine in the presence of pyridoxamine was dependent on the concentration of leucine. Extensive utilization of D isoleucine occurred when the leucine level was 0.4 mg. per tube but this utilization was completely blocked by increasing the leucine level to 2 mg. per tube. Although *L. arabinosus* can partially utilize D glutamic acid the extent of this utilization did not appear to be dependent on the form or amount of vitamin B<sub>6</sub> in the medium. It was found that vitamin B<sub>6</sub> is not the only factor which modifies the ability of the lactic acid bacteria to utilize D amino acids. *Streptococcus faecalis* R did not utilize D methionine in a medium buffered with acetate, but substantial utilization occurred in a medium buffered with citrate. The significance of these findings in relation to the microbiological determination of amino acids is discussed.

**Study of the oxidation of the labile methyl group of dietary methionine traced with C<sup>15</sup>.** COSMO G. MACFARLANE, JULIAN R. RACHITT (by invitation), NANCY CROSS (by invitation), JOSEPH P. CHANDLER, and VINCENT DU VIGLAUD, *Department of Biochemistry, Cornell University Medical College, New York City*. The amount of CO<sub>2</sub> in the expired air derived from the oxidation of the methyl carbon of dietary methionine (labeled with C<sup>15</sup> in the methyl group) was compared at two levels of methionine in the diet. Rats were allowed continuous access to a diet containing 0.1 per cent cystine, 0.2 per cent choline chloride, and either 0.6 or 1.2 per cent methionine. During the course of the experiment the animals were fed by stomach tube a single 2 gm. portion of the diet in which the ordinary methionine was replaced by radioactive methionine. On the low methionine diet 6%, and on the high methionine diet 25%, of the ingested methyl carbon appeared within 30 hours as radioactive CO<sub>2</sub> in the expired air. This difference in the proportion of the methyl group oxidized was due almost entirely to a difference in the relative rates of oxidation during the first 8 hours. On both diets, the rate of oxidation of the methyl group, as reflected in the C<sup>15</sup>O<sub>2</sub> content of the expired air, fell into two phases. The first of these (period of assimilation) was characterized by a rapid initial rise in content to the third hour, followed by a decline until about the eighth hour. The second phase (period of equilibrium) was initiated at this time by the establishment of a relatively low and constant rate of oxidation that persisted for the remaining 22 hours of the experiment. The influence of other dietary constituents on the oxidation of the methyl group of methionine is being investigated.

**Studies on the nitrogen metabolism of tomato using N<sup>15</sup>-labeled ammonium sulfate.** ROBERT MACVICAR (by invitation) and R. H. BURRIS, *Department of Biochemistry, University of Wisconsin*. Tomato plants were grown in sand culture with complete nutrient solution for six weeks, all nitrogen sources were then removed for ten days, at which time symptoms of nitrogen deprivation were appearing. 5.0 mg. of ammonium nitrogen as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> containing 30 atom % N<sup>15</sup> excess were supplied per plant at 7.00 A.M. Twelve hours later the plants were harvested, separated into aerial and root fractions, rapidly heated to 65° C, and dried. The dried tissue was then fractionated to obtain the principle nitrogenous fractions. The crude protein fraction was hydrolyzed and several amino acids and amino acid groups isolated from the hydrolysate. The N<sup>15</sup> content of these fractions was determined by mass spectrographic analysis and the atom per cent N<sup>15</sup> excess found to be

| Nitrogen fraction                          | Leaves and stem | Root |
|--|-----------------|------|
| Total nitrogen                             | 1 02            | 1 13 |
| Ethanol soluble                            | 1 74            | 3 89 |
| Water-soluble ethanol insoluble            | 0 49            | 0 77 |
| Crude protein hydrolysate                  | 0 53            | 0 79 |
| Neuberg precipitate of hydrolysate         | 0 56            | 0 64 |
| NH <sub>3</sub> and amide N of hydrolysate | 0 72            | 0 38 |
| Arginine                                   | 0 77            |      |
| Glutamic acid                              | 4 16            | 1 15 |
| Aspartic acid                              | 0 94            | 0 75 |
| Histidine                                  | 0 21            |      |
| Lysine                                     | 0 07            |      |

The data indicate appreciable incorporation of the absorbed nitrogen into tissue protein during the 12 hour period and confirms the observations of Vickery, *et al* in tobacco (*J Biol Chem*, 135 531, 1940). The markedly higher concentration of labeled nitrogen in the dicarboxylic amino acids, and particularly in glutamic acid, indicates a high order of reactivity of these compounds with the administered ammonium nitrogen. Histidine and lysine are apparently synthesized less rapidly.

**Effect of cytochrome C on the resistance of mice to anoxia.** GEORGE H. MANGUN, *Department of Laboratories, Henry Ford Hospital, Detroit*. Experiments were designed to determine the effect of cytochrome C on the resistance of male CFW mice to anoxia and asphyxia. Animals injected intravenously via the tail vein with cytochrome C and an equal number of control animals injected with an equal volume of saline were placed together in pairs in a closed system of 500 cc capacity 2 to 6 hours after injection, with or without absorption of CO<sub>2</sub>, maintaining atmospheric pressure by the introduction of nitrogen. With dosages of 50-100 mg/kg of cytochrome C, 6 controls and 6 cytochrome C injected animals exhibited symptoms and died simultaneously. At 200 mg/kg, results were somewhat variable, with 4 of the 6 cytochrome C injected animals dying 2-4 minutes earlier. At dosages of 300-450 mg/kg, 8 cytochrome C injected animals uniformly exhibited symptoms and succumbed before the controls. These experiments indicate that cytochrome C is unable to protect mice against the effects of a primary oxygen deficit. They are being repeated with other preparations of cytochrome C.

**The chemical composition of human thoracic aortas.** ROGER W. MARSTERS (by invitation), JACK R. LEONARDS (by invitation), and VICTOR C. MYERS, *Department of Clinical Biochemistry, Western Reserve University, Cleveland Ohio*. Sixty-four human thoracic aortas have been analyzed for water, creatine, collagen, elastin, cholesterol, calcium, and phosphorus. The water content showed no significant change with age but it was decreased

in severely arteriosclerotic specimens. Both creatine (as an index of smooth muscle) and elastin tended to decrease with age, while the collagen content increased, however, the differences are not statistically significant. The most outstanding changes were in the cholesterol, calcium and phosphorus contents of the aortas, marked increases in these three constituents occurring with age. There is a good correlation between the results of chemical analysis and the pathological diagnosis at autopsy. With increasing arteriosclerosis there are marked increases in cholesterol, calcium, and phosphorus content. In most cases these three constituents increased in a parallel manner but occasionally the arteriosclerotic deposits were predominantly cholesterol.

**Enzymatic destruction of cholesterol by rat liver extract in vitro.** WALTER MARX and MORRIS LIPSETT (introduced by HARRY J. DEUEL, JR.), *Department of Biochemistry, University of Southern California Medical School, Los Angeles*. It is known from balance experiments (Schoenheimer, Page and their co-workers) that the animal organism is able to dispose of cholesterol, when relatively large quantities are fed with the diet. The possibility was considered that this destruction of cholesterol is of an enzymatic nature, and it was attempted to demonstrate *in vitro* such an enzyme capable of destroying cholesterol. Various homogenates and extracts of rat liver and spleen were incubated with cholesterol under different conditions. Results of experiments using tissues from normal rats were equivocal. Liver extracts were then made from rats which had been fed a diet containing 1% cholesterol and 0.5% bile salt for periods of several weeks or months. The extracts were made using saline or phosphate buffer at pH 6 to 7, and they contained appreciable quantities of cholesterol. When such extracts were incubated at 37°C for periods of 12 to 16 hours, a significant decrease in the concentration of total cholesterol.

**The hydrolysis of nuclear proteins by cathepsins I.** Calf thymus cathepsin MARY E. MAYER and ANTOINETTE GRACO (by invitation), *National Cancer Institute, Bethesda 14, Md*. The proteins of calf thymus, which were insoluble in 0.325M KCl and were sedimented at 2,000 r.p.m., were hydrolyzed by the cathepsins prepared from calf spleen and calf thymus with optima at pH 4.0 and at pH 6.0-6.5. The proteinase activity at pH 4.0 was activated by cysteine while that at the higher pH was not. This protein fraction yields both nucleic acid and histone abundantly. It contained approximately 15% nitrogen and 2 percent phosphorus. The nuclear protein or nucleohistone fraction of calf thymus, separated by the usual method of solution in weak alkali and precipitation with acetic acid, was hydrolyzed at one pH optimum of

pH 4.5-5.0 The thymus histone and clupein, the protamine of herring sperm, were also hydrolyzed by calf spleen and thymus cathepsins

**Vitamin A deficiency and diet composition**  
JEAN MAYR (by invitation) and W. A. KREHL, *Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University* Sprague Dawley weanlings of uniform weight were fed vitamin A free, synthetic diets, controls were kept for each group. Three diets were designed to study the influence of the protein level (10, 25 and 60% casein) on the growth and survival time of the deficient animals. Two high fat diets (purified lard and corn oil) were used to demonstrate the possible influence of saturated and unsaturated fats. Similarly dextrin was compared with sucrose. When casein replaces sucrose isocalorically, survival time varies inversely with the protein level and bears no direct relationship to the maximum weight attained. The difference in the average weights of the three control groups at 10 days was not statistically significant. Fats were found to be protective against vitamin A deficiency, as evidenced by an increased survival time. The average maximum growth of the deficient animals on hard fat was considerably and consistently better than on corn oil, although no significant growth difference was seen in the controls. The replacement of sucrose by dextrin did not result in any change. The authors have concomitantly demonstrated that vitamin C deficiency was an integral part of the vitamin A deficiency syndrome in the rat. The modification of the picture given above by addition of ascorbic acid is now being studied.

**Pituitary-protein-bound iodine and the Plummer treatment of hyperthyroidism** J. F. MCCLENDON and W. C. FOSTER (by invitation), *Hahnemann Medical College, Phila.* and J. W. CAVETT (by invitation), *Salisbury's Laboratories, Charles City, Iowa*. For several weeks 74 chickens were fed 0.2% thiouracil, 71 chickens received 4 cc Lugol's solution per gallon drinking water and 71 were controls. The pituitaries of the controls contained an average of 0.052 micrograms of protein-bound iodine, PBI, (3 micrograms per gram fresh). The pituitaries of chickens fed thiouracil averaged 0.044 microgram PBI (2.5 micrograms per gram) and the pituitaries of chickens given Lugol's averaged 0.077 microgram PBI (3.8 micrograms per gram). The desiccated thyroids of the controls contained 0.199% PBI, those of the thiouracil-fed 0.0253% PBI and those of Lugol's-fed 0.372% PBI. This excess PBI in the thyroid apparently was not passed into the circulation as the chickens did not lose weight. Therefore we believe that the excess PBI in the pituitary has not migrated from the thyroid but is formed in situ (as McClendon and Foster postulated for sheep, *Fed. Proc.* 6:275) inside the pituitary, where it suppresses the for-

mation of thyrotropin. Since the PBI cannot exceed the total iodine and the pituitary is more than 80% water, our values are 5 times those of Closs who found 1 to 2 micrograms total iodine per gram dry pituitary. He spoke of "possibility of contamination" of commercial preparations 50 times his values. (It should be noted that U. S. P. thyroid contains 2000 micrograms per gram.) We interpret the variations of PBI in the pituitary as being formed during life and not due to contamination of the sample.

**Methionine in the growth of the malarial parasite, *Plasmodium knowlesi*** RALPH W. MCKEE and QUEENTIN M. GELMAN (by invitation), *Departments of Biological Chemistry and of Comparative Pathology and Tropical Medicine, Harvard Medical School, Boston, Massachusetts*. *Plasmodium knowlesi* requires the addition of methionine for its *in vitro* growth and multiplication (*Fed. Proc.* 6, 276, 1947). This means that the parasite needs more methionine than it can obtain from the host red cell and from its immediate environment. Since this requirement is so critically important, it seemed desirable to determine, (1) possible changes in the amount of free methionine in the circulating plasma of the host animal, *Macaca mulatta*, and what effect this might have on the *in vivo* growth of the parasites and (2) the effects of methionine analogs and methyl acceptor compounds on parasite growth and multiplication both *in vitro* and *in vivo*. The *P. knowlesi* infection in our monkeys is controlled by fasting the animal (not by subjecting the animal to lowered temperature, another stress condition) and is reversed by giving dl-methionine or para-aminobenzoic acid. This control of the parasitemia is not reversed by the oral administration of a body weight-maintenance level of sucrose or of sucrose plus injected ascorbic acid. Diets complete, except for methionine, are being tested. The plasma levels of methionine under these conditions are being determined. The *in vitro* growth of *P. knowlesi* is markedly inhibited by methionine and ethionine and the inhibition is overcome by the addition of extra methionine. The antagonist/methionine ratios are about 10/1 and 1/1, respectively. Guanidoacetic acid, an acceptor of methyl groups from methionine, is being tested in monkeys, both for its effect on parasite growth and on the plasma level of methionine.

**A crystalline compound of  $\beta$ -Lactoglobulin with dodecyl sulfate** T. L. McMEIKIN, B. D. POLIS (by invitation), L. S. DELLA MONICA (by invitation) and J. H. CUSTER (by invitation), *Eastern Regional Research Laboratory, Philadelphia 18, Pa.* A crystalline derivative of  $\beta$ -lactoglobulin containing two equivalents of dodecyl sulfate has been prepared. The compound is apparently undissociated, since the dodecyl sulfate is not removed

by barium ion. The iso electric point of the dodecyl derivative is slightly more acid than that of  $\beta$  lactoglobulin, as shown by its mobility. Moreover, a salt solution of the dodecyl derivative of  $\beta$  lactoglobulin has a pH of 5.06, as compared with 5.18 for  $\beta$  lactoglobulin. The solubility of the dodecyl derivative is about one-half as large in water and one third as large in dilute salt solutions as that of  $\beta$  lactoglobulin. The titration curves of both these substances are essentially the same on the acid side of the iso electric point, while on the alkaline side the titration curve of the dodecyl derivative indicates a greater alkali-combining capacity. This finding is in agreement with the increased mobility obtained with the dodecyl derivative, as compared with that of  $\beta$  lactoglobulin on the alkaline side of the iso electric point. The dodecyl derivative has the same amount of amino nitrogen as  $\beta$  lactoglobulin (on a total N basis), as indicated by the Van Slyke method. This dodecyl derivative did not give a test for sulfhydryl groups. Efforts to remove the two equivalents of dodecyl sulfate from  $\beta$  lactoglobulin have failed, indicating that previous reports of the complete removal of this substance from proteins are in error, since the mobilities of the proteins after treatment with dodecyl sulfate and subsequent removal are invariably different from those of the untreated proteins.

**An egg yolk protein containing 10% phosphorus**  
DALE K. MECHAM (by invitation) and HAROLD S. OLCOTT, *Western Regional Research Laboratory, Albany, Calif. Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture*. A protein preparation containing 10% phosphorus is obtainable from egg yolk as follows. Diluted yolk is centrifuged (Sharples). The insoluble fraction is lyophilized, extracted with ether, and dispersed in 5% sodium sulfate. Addition of sodium sulfate to a concentration of 36% precipitates the bulk of the proteins. The phosphoprotein is precipitated from the remaining solution with copper acetate. The precipitate is dissolved at pH 2 and reprecipitated at neutrality. After further purification steps, the copper is removed by dialysis against citrate buffer (pH 5). One preparation appeared to be 90% homogeneous by electrophoretic analysis. The following properties are typical: nitrogen, 12%, phosphorus, 10%, sulfur, none, number average molecular weight (osmotic pressure), 18,000 to 25,000. Titration data indicate that the phosphorus occurs as ortho phosphate ester. Sufficient serine is present to account for at least 50% of the phosphorus, and the presence of *o* phosphate esters of serine residues is further suggested by a marked destruction of serine during acid hydrolysis and by the development of increased ultra violet absorption, characteristic of dehydroalanine residues, after alkaline dephosphorylation. The phospho-

protein resists digestion by whole pancreas extract but becomes digestible after removal of the phosphate groups with phosphatase (orange or grapefruit). At least 40%, and possibly considerably more, of the total protein phosphorus content of egg yolk can be accounted for in the new phosphoprotein, for which the name "phosvitin" is proposed.

**Enzymatic hydrolysis of acetopyruvic acid**  
ALTON MEISTER (by invitation) and JESSE P. GREENSTEIN, *National Cancer Institute, National Institute of Health, Bethesda, Maryland*. Acetopyruvic acid ( $\alpha, \gamma$  diketovalerianic acid) is rapidly hydrolyzed to yield nearly equivalent amounts of pyruvic acid equally well in aerobic or anaerobic digests with aqueous extracts of rat liver and kidney. The optimum pH for the reaction is about 7.0. Prolonged dialysis of the extracts does not appreciably affect the reaction. There is no detectable acetoacetate formation. The resulting pyruvic acid is measured as the 2,4-dinitrophenylhydrazone and has been identified by isolation. Rat spleen, pancreas, brain, skeletal and cardiac muscle, testes, intestine, stomach, lung, and primary hepatoma show negligible activity. Acetopyruvate accelerates the desamidation of glutamine by rat liver to nearly the same extent as does the equivalent amount of pyruvate. However, no acceleration of glutamine desamidation by acetopyruvate was noted in the rat hepatoma, thus indicating that the diketo acid itself is not active in the glutamine system. Freshly prepared acetopyruvic acid was employed as the purified sodium salt. This salt, the free acid obtained therefrom, and the ethyl ester of the acid all possess a characteristic absorption in the ultraviolet with a maximum at 2950 Å for the Na salt, 2850 Å for the free acid, and 2900 Å for the ester.

**Inhibition of hyaluronidase by hydroquinones and quinones**  
KARL MEYER and CHARLES RAGAN (by invitation) with the technical assistance of HANNAH WEINSHELBAUM, *Departments of Ophthalmology and Medicine, School of Medicine, Columbia University, New York*. The inhibition by salicylate of the spreading reaction in animals and in man (Guerra, F. J. *Pharm. Exp. Therap.* 87, 1943, 1946) has been confirmed. However, in vitro salicylate in therapeutic levels had no effect on hyaluronidase activity (Meyer, K. *Physiol. Revs.* 27, 335, 1947; Pike, R. M. *Science* 105, 391, 1947; Dorfman, A. et al. *Proc. Soc. Exp. Biol. and Med.* 64, 357, 1947) but did inhibit in concentrations which denatured the protein. The urine of patients on salicylate therapy was fractionated and the fractions tested for inhibition of hyaluronidase. A potent inhibitor was found in the recrystallized salicylic acid fractions, but synthetic salicylic acid had no effect. The inhibition proved to be due to gentisic and gentisuric acids. Synthetic gentisic

acid after short incubation with hyaluronidase gave strong inhibition which could be prevented by hyaluronate. This observation is in agreement with the statement that 2,5 benzoquinone carboxylic acid inhibits hyaluronidase (Lowenthal, J and Gagnon, A. *Science* 105 618, 1917). The following hydroquinones and quinones strongly inhibited hyaluronidase in 0.0005 mol concentration: hydroquinone dicarboxylic acid (25%), synthetic gentisic acid (14%), homogentisic acid (59%) and polyporic acid (93% inhibition). The significance of the findings will be discussed.

**The free energy of phosphorylation** OTTO MEYERHOFF and PLILR OESPER (by invitation). *Department of Physiological Chemistry, School of Medicine, University of Pennsylvania*. From equilibrium measurements by means of phosphatase (Schmidt's intestinal phosphatase)  $\Delta F^\circ$  for hydrolysis of primary alcoholic esterphosphate in hexosephosphates, D 3 phosphoglyceric acid, etc. is found to be  $-300$  to  $-500$  cal. By means of the equilibrium of the reaction: phosphopyruvate + ADP  $\rightleftharpoons$  pyruvate + ATP,  $\Delta F^\circ$  (from left to right) is found to be about  $-3000$  to  $-3500$  cal. The same  $\Delta F^\circ$  of transphosphorylation obtains from 1,3-diphosphoglyceric acid to ATP. From these and other equilibria, which were measured formerly by the authors and others, four classes of phosphate compounds can be distinguished with regard to the  $\Delta F^\circ$  (25°C, pH 8) of phosphorylation, they are in decreasing order:

- 1 Acylphosphates and enolphosphates,  $\Delta F^\circ$  around  $-14000$  cal
- 2 Pyrophosphates and guanidinophosphates,  $\Delta F^\circ$  around  $-10500$  cal
- 3 Carbonylphosphate (Glucose-1-phosphate),  $\Delta F^\circ$  around  $-2000$  cal
- 4 Primary alcoholic phosphates,  $\Delta F^\circ$  around  $-400$  cal

The bearing of these data for the energy transfer will be discussed.

**Loss and restoration of rat liver enzymes (activity) related to dietary changes in liver protein** LEON L. MILLER. *Department of Biochemistry, The Jefferson Medical College, Philadelphia, Pa.* The activity of five enzymes (catalase, alkaline phosphatase, xanthine dehydrogenase, cathepsin, and arginase) has been measured by using homogenates of livers from Sherman strain rats. When compared with the livers of normal controls on a 25% casein diet adequate in vitamins, the livers of animals on a non protein diet for 8 to 18 days show a loss of activity of all the enzymes studied. The loss of activity is greatest when expressed in terms of units of activity per 100 grams of initial body weight, but is highly significant when expressed in units per gram of liver protein ( $N \times 6.25$ ). The loss of enzyme activity is most probably the result of the loss of enzyme protein per se. On this basis

presumably vital enzyme proteins are not spared, but are lost along with liver cell protein in general. A small group of rats was fed a 25% casein diet after 30 days on a 6% casein diet followed by 5 to 9 days on the non protein diet. In most of the rats there was prompt restoration toward normal of liver protein and enzyme protein (i.e. activity).

**Utilization of pyruvate by heart ventricle *in vitro* as a function of time** O. N. MILLER (by invitation), R. L. OLSON (by invitation) and F. J. STARE. *Department of Nutrition, Harvard School of Public Health and Department of Biological Chemistry, Harvard Medical School, Boston*. The rate of pyruvate utilization by cells *in vitro* has been determined for a variety of animal tissues, eggs, and microorganisms by a number of investigators. The object of the present study was to determine the effect of the length of the period of measurement and the time of substrate addition upon the rate of pyruvate disappearance and lactate production in slices of ventricle from cardiac muscle of well nourished rats and ducks. Slices of ventricle were bathed in phosphate saline containing 5 mM/L of pyruvate for varying intervals in a standard Warburg apparatus at 37° in an atmosphere of oxygen. In some experiments pyruvate was initially present, in others it was added after different intervals. Pyruvate and lactate were determined chemically and the rates of pyruvate disappearance and lactate production expressed as (Q). The following table gives the results obtained.

| Time of measurement | -Q <sub>pyruvate</sub> | +Q <sub>lactate</sub> |
|---------------------|------------------------|-----------------------|
| <i>minutes</i>      |                        |                       |
| 5-15                | >20                    | >10                   |
| 15-30               | 14-20                  | 5-10                  |
| 30-60               | 8-14                   | 3-5                   |
| 60-120              | 5-8                    | 2-3                   |
| 120-240             | <5                     | <2                    |

The values obtained with rat and duck ventricle are included together in the above ranges although, in general, the rat values were higher than the duck values. Comparable decreases in pyruvate utilization were obtained by adding pyruvate after given intervals of incubation without added substrate.

**Chromatography of amino acids** Colorimetric ninhydrin method for analysis of the effluent STANFORD MOORE (by invitation) and WILLIAM H. STEIN. *Rockefeller Institute for Medical Research, New York City*. The reaction of ninhydrin with  $NH_2$  groups to give diketohydrindylidene diketohydrindamine has been utilized as the basis for a colorimetric determination of amino acids and related compounds in effluent samples from starch chromatograms. The lack of reproducibility of the color yield from a given amino acid in the ninhydrin reaction, and the marked deviations from Beer's law which have been observed, are overcome by the



incorporation of hydrindantin or stannous chloride in the reagent solution to eliminate oxidative side reactions. Although under these conditions the color yield from a given amino acid is constant, the different amino acids do not all give the same percentage yield of the blue product. This fact does not prevent the accurate use of the method in chromatographic work in those cases where the individual amino acids are separated from one another by the fractionation process. The ninhydrin reaction is carried out at pH 5 and 100°C. The absorption maximum of the colored product is at 570m $\mu$  for all the  $\alpha$  NH acids (except cysteine), whereas with the  $\beta$  naphthoquinone sulfonic acid reaction, the maxima are similar but not identical. On individual amino acids the accuracy is  $\pm 2$  per cent with samples containing 3 micrograms of  $\alpha$  NH-nitrogen.

The color development is obtained with a variety of compounds containing NH groups, including amino acids, peptides, primary amines, and ammonia. For chromatographic work the generality of the reaction extends its usefulness. For work with unfractionated biological material the lack of specificity is a disadvantage.

The purification and crystallization of phosphoglucomutase. VICTOR A. NAJJAR, *Department of Biological Chemistry, Washington University Medical School*. Perfused rabbit muscle is passed through a meat grinder and extracted with two volumes of water (I), adjusted to pH 5 with molar acetic acid, heated to 65°C and filtered. The filtrate (II) is made to 0.65 ammonium sulfate saturation. The precipitate is dissolved in acetate buffer pH 5.0 (III) then heated to 63°C and centrifuged. The supernatant (IV) is raised to an ammonium sulfate concentration of 0.50 and precipitate discarded. The concentration is raised gradually to 0.55 where crystals appear after a few hours. The saturation is further raised to 0.60 and allowed to stand 24 hours. The 0.50-0.60 crystals (V) are then separated, dissolved in acetate buffer and recrystallized with ammonium sulfate. The crystalline fraction between 0.55-0.60 (VI) is approximately 90% pure and that between 0.60-0.65 (VII) is electrophoretically homogenous at pH 5.0.

|                                 | Fractions |     |     |      |     |    |     |
|---------------------------------|-----------|-----|-----|------|-----|----|-----|
|                                 | I         | II  | III | IV   | V   | VI | VII |
| Activity in units/mg protein    | 0.5       | 3.0 | 6.0 | 14.0 | 21  | 23 | 26  |
| Phosphoglucomutase protein (mg) | 400       | 200 | 150 | 140  | 130 | 19 | 29  |

The enzymatic hydrolysis of glutathione. KAZUO NAKAMURA (by invitation) and FRANCIS BINKLEY, *Laboratory for the Study of Hereditary and*

*Metabolic Disorders and the Departments of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City, Utah*. It has been found that glutathione is hydrolyzed to cysteinylglycine and glutamic acid by an enzyme of kidney tissue. This enzyme is relatively stable to heat and is activated by magnesium ions. The enzyme responsible for the hydrolysis of cysteinylglycine to cysteine is found in liver and muscle as well as in the kidney. This enzyme is labile to heat and is strongly inhibited by calcium or magnesium ions. Desiccated hog kidney (Viobin Corporation) is a good source of the enzyme responsible for the hydrolysis of glutathione to cysteinylglycine; considerable purification of this enzyme has been achieved. The purified enzyme has been utilized in the estimation of glutathione in whole blood and in homogenates of tissue. The cysteinylglycine is measured by the method of Sullivan and Hess (*J. Biol. Chem.*, 116, 221 (1936)) for cysteine. Glutathione, added to whole blood or homogenates of tissues, may be quantitatively recovered by this method.

Blood sugar changes following the administration of lactose in raw and evaporated milk. SAMUEL NATELSON (by invitation), BENJAMIN KRAVER and MARVIN SHERMAN (by invitation), *Pediatric Research Laboratory, Jewish Hospital of Brooklyn*. The same infants on successive days (3rd and 4th day after birth) show fairly close correlation in carbohydrate tolerance curves when the same carbohydrate is administered as measured by the concentration of reducing sugar in blood. The following formulae were compared in the same infant by measuring carbohydrate tolerance curves on successive days: a) Lactose in evaporated milk formula vs. Lactose in raw cow's milk formula, b) Starch hydrolysate (Cartose) in evaporated milk formula vs. Starch hydrolysate in raw cow's milk formula, c) Raw mother's milk. Lactose in raw milk formula shows higher peaks and more rapid return to the fasting level than Lactose in evaporated milk formula. Starch hydrolysate in evaporated milk formula shows higher peaks and a more rapid return to the fasting level than lactose in the same formula, but improvement is observed when the starch hydrolysate is administered in raw milk. Raw mother's milk per se shows higher peaks than any of the above formulae. The time required for return to fasting level was the same as for lactose in raw cow's milk. These observations may explain our statistical growth studies which show improved weight curves for infants, in the first seven days of life on breast milk supplemented with starch hydrolysate (Cartose) over evaporated milk supplemented with starch hydrolysate.

Response of citric acid levels to administration of glucose. SAMUEL NATELSON, JOSEPH B. PINCUS and JULIUS K. LUGOVY (introduced by ALBERT

E SOBEL) *Department of Biochemistry of the Jewish Hospital of Brooklyn* Following the administration of glucose orally the citric acid level, in serum, drops slowly for the first thirty minutes and then drops sharply to a minimum point (25-35% lower than the fasting level) about 110-200 minutes after glucose administration. A gradual return, after 5-6 hours, to the fasting level is then observed. The minimum for citric acid corresponds with the period of most rapid drop in glucose level. Injection of insulin, without glucose administration, causes a drop in citric acid serum level. Most diabetics exhibit a citric acid response which resembles the normal. Certain convulsive children and diabetics show an abnormal citric acid response. This abnormality takes the form of a rising level of citric acid which does not fall during the test period. Another form of abnormality was a sharp rise for the first hour followed by a lowering of the citric acid level to a minimum below the fasting level. An *in vivo* relationship between the intermediate metabolism of glucose and citric acid is therefore indicated, which may be disturbed in certain pathological conditions.

**The enzymatic liberation of pantothenic acid**  
J B NEILANDS (by invitation) and F M STRONG  
*Department of Biochemistry, University of Wisconsin, Madison, Wisconsin* The purified coenzyme for acetylation (*J Biol Chem* 162, 743, 1946) was found by Lipmann and his associates (*J Biol Chem* 167, 869, 1947) to contain pantothenic acid. In this form the pantothenate is unavailable to *L casei* although it may be liberated by the simultaneous action of a purified alkaline intestinal phosphatase and a pigeon-liver enzyme. The use of these enzyme treatments in the preparation of samples for pantothenic acid assay has been compared with the mylase P digestion method (*Arch Biochem* 9, 251, 1946). In general the new procedure gives approximately a 3 fold increase in the apparent pantothenic acid level, although thirty-five fold increases have been obtained by allowing the enzymes to act on a boiled extract of chicken liver. The latter was prepared immediately after death. The pigeon-liver enzyme has been found to be replaceable by a chicken-liver enzyme, the latter is a more convenient source for routine assay work. It would appear that a large share of the pantothenic acid present in a wide variety of foods and other biological materials exists in the combined form, and has not been included in most of the previously reported assay values.

**Kinetics and inhibition of carboxypeptidase activity**  
HANS NEURATH and ELAINE ELKINS (by invitation) *Department of Biochemistry, Duke University School of Medicine, Durham, N C* Independent determinations of the proteolytic coefficient ( $C = k/E$ ) for the hydrolysis of carboxybenzoylglycylphenylalanine (CGP) by carboxypep-

tidase, in 0.05 M substrate solutions, have yielded a value of about  $C = 10$ . Present measurements reveal a large dependence of the first order reaction constant,  $k$ , on substrate concentration (DL-CGP). The corresponding proteolytic coefficients approach a minimum value of  $C = 5$  above 0.075 M substrate (with respect to L-CGP) and a maximum value of  $C = 53$  below 0.001 M substrate. A similar trend was observed with solutions of L-CGP. The data fit closely the equations of Lineweaver and Burk. None of the reaction products, i.e. carboxybenzoylglycine and L-phenylalanine, inhibits hydrolysis nor does D-CGP. However, hydrolysis is largely inhibited by D-phenylalanine. The possibility of optical inversion during enzymatic hydrolysis has been excluded by the observation that phenylalanine isolated from the enzymatic hydrolysate of L-CGP is not oxidized by D-amino acid oxidase. It appears, therefore, that the dependence of reaction rates on substrate concentration is unrelated to the inhibition by D-phenylalanine. This conclusion is further corroborated by the findings that the latter inhibition is of the non competitive type. While D-lysine is not inhibitory, the extent of inhibition by other D-amino acids decreases in the order: phenylalanine > histidine > valine = isoleucine. It is noteworthy that these D-isomers are inhibitory despite the fact that peptides containing some of the corresponding L-isomers are not specific substrates for carboxypeptidase.

**Estrogenic activity of doisylnolic acid and related compounds**  
HAROLD J. NICHOLAS (by invitation), SIDNEY A. THAYER and EDWARD A. DOISY  
*Laboratory of Biological Chemistry, Saint Louis University School of Medicine, Saint Louis, Missouri* Since Miescher's discovery of the pronounced estrogenic activity of 1-ethyl-2-methyl-7-hydroxy-1,2,3,4,9,10,11,12-octahydrophenanthryl-2-carboxylic acid (doisylnolic acid) and some related compounds, we have reinvestigated some of these derivatives in our laboratory. Doisylnolic acid and the 7-benzoate methyl ester of this compound have been assayed in sprayed rats and mice. Similarly bisdehydrodoisylnolic acid and its methyl ether have been assayed. We find that doisylnolic acid injected subcutaneously is less active than estrone in sprayed rats. However, this acid is more active than estrone administered orally to sprayed rats. It is considerably less active than estrone (approximately 1/150 as active) by subcutaneous injection in sprayed mice, an observation which agrees with our earlier assay of this substance. Doisylnolic acid possesses about the same order of activity as estrone when given orally to mice. These data again emphasize the difficulty in attempting to compare the activity of one compound with another estrogenic material using only one species of animal. From the mixture resulting from

fusion of estrone with potassium hydroxide we have isolated doisylnolic acid, estrone and another steroid insoluble in carbonate

**Respiratory metabolism in yeast and coenzyme A levels** Effect of phenyl panthenone on coenzyme A synthesis G DAVID NOVELLI (by invitation) and FRITZ LIPMAN *Biochemical Research Laboratory, Massachusetts General Hospital, and the Department of Biological Chemistry, Harvard Medical School, Boston* Samples of yeast, high and low in coenzyme A (Co A), were obtained from pantothenic acid deficient yeast by pre treatment, in a glucose-phosphate medium, with and without pantothenic acid In this manner samples could be compared containing an average of 350 and 100 units of Co A respectively The respiration of acetate, ethanol and glucose was followed, increase in Co A corresponded with a doubling to tripling of the respiratory rate with acetate and ethanol and about 30% increase with glucose In the deficient samples an accumulation of acetic acid occurred with ethanol and glucose Malonate depressed the stimulated respiration of acetate while there was little effect in the deficient sample Preliminary results have been obtained in a study with dried yeast of citrate synthesis from acetate and oxalacetate The described yeast system offers an opportunity to study the mode of action of pantothenic acid antimetabolites Mainly phenyl panthenone (Wooley et al *J Biol Chem*, 159 263, 1945) was studied Its presence, together with pantothenic acid, in the pre treatment period, was found to suppress completely Co A synthesis yielding a preparation with low acetate oxidation When added, however, after the enrichment period, acetate oxidation was unaffected or rather stimulated by addition of phenylpanthenone The observation indicates the action of phenylpanthenone to be due to a blocking of the synthesis of Coenzyme A from pantothenic acid

**Effect of biotin deficiency upon the respiration of cardiac muscle in ducklings** R E OLSON (by invitation), O N MILLER (by invitation), and I J STARR *Department of Nutrition, Harvard School of Public Health and Department of Biological Chemistry, Harvard Medical School, Boston* A number of recent reports have indicated that biotin functions in metabolism to facilitate the reversible carboxylation of pyruvic acid to oxalacetic acid If the failure to accomplish this reaction were a major biochemical lesion in biotin deficient tissues it might be expected that the rate of utilization of pyruvate as well as intermediates of the tricarboxylic acid cycle would be depressed To test this hypothesis we have determined the rate of oxygen consumption of slices of ventricle from hearts of biotin deficient and normal ducks without added substrate and with added pyruvate and succinate The rate of disappearance of pyruvate during the period of respiration was determined chemically and the rate of succinate metabolism was measured by determining the rate of radioactive  $\text{CO}_2$  production from carboxyl labeled succinate during the period of respiration The oxygen consumption of biotin deficient heart ventricle is markedly depressed Without added substrate it is reduced 30%, with added pyruvate 27% and with added succinate 23% The rate of disappearance of pyruvate to non-lactate products is reduced 65% and the rate of  $\text{CO}_2$  production from succinate is reduced 45% The injection of biotin to deficient birds restores these values approximately to normal

**Further studies on the effect of cysteine and histidine on the production of cobalt polycythemia** JAMES M ORTEN *From the Department of Physiological Chemistry, Wayne University College of Medicine, Detroit* Earlier studies in this laboratory have demonstrated that the oral administration to rats of cysteine or of histidine significantly inhibit the production of polycythemia by cobalt also given orally On the other hand, methionine and choline in equivalent amounts had no detectable effect Since both cysteine and histidine form less soluble complexes with cobalt than inorganic cobalt salts, the question was raised as to whether the inhibiting effect of these two amino acids might be explained on the basis of a decrease in the intestinal absorption of cobalt In order to study this question further, the complexes of cobalt with cysteine and with histidine have been prepared and injected subcutaneously into rats in amounts equivalent to 10 mg cobalt per day Control rats were injected the same amount of cobalt as the sulfate A 12 week period of observation was employed during which time the hemoglobin content of the blood was determined biweekly The data obtained thus far indicate that cobalt injected as the cysteine complex does not produce a polycythemia Studies with the histidine complex are in progress The relation of these observations to the possible mechanism of cobalt action in increasing hemopoiesis will be discussed

**Peptidase increase during growth of the salivary glands of the fly, *Drosophila melanogaster*** ELIZABETH K PATTERSON, MARJORIE C DACKERMAN, and JACK SCHULTZ (introduced by THEODORE F LAVINE) *Lankenau Hospital Research Institute and Institute for Cancer Research, Philadelphia, Pa* Peptide splitting enzymes have been studied during growth of the salivary glands of various genetic stocks of *Drosophila melanogaster* Using Linderström-Lang Holter micro techniques extracts of the glands are found to have the ability to split dipeptides, tripeptides, leucylglycine, leucylglycylglycine, and triglycine in order respective to their activity Action of activators and inhibitors, pH activity, concentration, and time curves show the

Peptidase increase during growth of the salivary glands of the fly, *Drosophila melanogaster* ELIZABETH K PATTERSON, MARJORIE C DACKERMAN, and JACK SCHULTZ (introduced by THEODORE F LAVINE) *Lankenau Hospital Research Institute and Institute for Cancer Research, Philadelphia, Pa* Peptide splitting enzymes have been studied during growth of the salivary glands of various genetic stocks of *Drosophila melanogaster* Using Linderström-Lang Holter micro techniques extracts of the glands are found to have the ability to split dipeptides, tripeptides, leucylglycine, leucylglycylglycine, and triglycine in order respective to their activity Action of activators and inhibitors, pH activity, concentration, and time curves show the

enzyme splitting alanyl-glycine to be a typical intracellular peptidase. Alanyl-glycine is used as the substrate in the subsequent experiments on single glands. The larval salivary glands reach their maximum size in the early prepupa and then disintegrate. Cell division stops in the embryo and therefore growth of a constant number of cells of like physiological function may be studied. Comparison of different stocks attaining diverse nitrogen levels at maximum growth shows the peptidase content of the glands to be proportional to their nitrogen content. During growth the ability of the glands to split alanyl-glycine increases with their nitrogen content. As the glands break down, a sharp decrease in splitting ability occurs. The results show a correlation of peptidase content with growth of the cells and not with their breakdown.

The isolation of pregnanol-3( $\alpha$ )-one-20, Comp'd Y ( $C_{27}H_{46}O_2$ ) and Comp'd Z ( $C_{27}H_{44}O_2$ ) from the bile of pregnant cows W. H. PEARLMAN, *Department of Biochemistry, Jefferson Medical College, Philadelphia*. That batch (31.0 liters) of bile from which estrone had previously been isolated (Pearlman et al., *J. Biol. Chem.*, **170**, 173 (1947)) was further investigated. The ether-soluble neutral material of unhydrolyzed bile was repeatedly partitioned between petroleum ether and 90% methanol. The latter fraction was separated into ketonic and non-ketonic moieties with the aid of Girard's reagent T. The ketones were treated with succinic anhydride to obtain the alcoholic ketonic fraction which, following the removal of a small quantity of digitonin-precipitable material, was chromatographed. There were obtained by these procedures:

5 mg. of pregnanol 3( $\alpha$ )-one-20. It was identified by its melting-point, specific optical rotation and C,H analysis; a diacetyl derivative was also prepared and similarly identified. The non-ketonic fraction, on treatment with ethyl acetate, yielded 142 mg. of a white crystalline product, m.p. 200-208° which proved to be a mixture and was therefore chromatographed. Comp'd Z, m.p. 229-229.5° [ $\alpha$ ]<sub>D</sub> = -9° (dioxan) was thereby obtained. Found 78.15% C, 11.07% H,  $C_{27}H_{44}O_2$ , calculated, 78.03% C, 11.03% H. Comp'd Y, m.p. 234-236° [ $\alpha$ ]<sub>D</sub> + 19° (dioxan) was also obtained. An additional 33.0 liters of bile was obtained from pregnant cows and worked up as before. There were obtained 20 mg. of Comp'd Z, m.p. 228.5-229° [ $\alpha$ ]<sub>D</sub> -2° (ethanol), found 78.12% C, 11.05% H. There were also obtained 16 mg. of Comp'd Y (rectangular plates from alcohol), m.p. 235-236° [ $\alpha$ ]<sub>D</sub> +20° (abs. ethanol). Found 78.73% C, 11.25% H,  $C_{27}H_{46}O_2$ , calculated, 78.69%, 11.33% H. Comp'ds Y and Z are non-digitonin precipitable. Comp'd Y is not identical with pregnanediol-3( $\alpha$ ),20( $\alpha$ ). Structural elucidation of these products is in progress.

**Preliminary observations on the renal excretion**

**of radioiodine after administration of tracer doses** MARSHALL H. POWER, WILLIAM C. MCCONAHEY, JR. (by invitation), F. RAYMOND KETTING, JR. (by invitation) and JOSEPH BERKSON (by invitation). *Divisions of Biochemistry, of Medicine and of Biometry and Medical Statistics, Mayo Foundation and Mayo Clinic, Rochester, Minnesota*. Tracer doses equivalent to about 100 micro-curies of radioactive iodide ( $I^{131}$ , Oak Ridge Operations, Atomic Energy Commission), together with 100 micrograms of nonradioactive sodium iodide, have been ingested by human beings. Subsequently samples of blood plasma and of urine, collected at suitable intervals, have been analyzed for their content of  $I^{131}$ . Plasma clearances of  $I^{131}$ , calculated from these data by the conventional procedure, that is, by multiplying the ratio of the concentrations of  $I^{131}$  in urine and plasma by volume of urine per minute range from about 40 cc. per minute in normal persons down to 15 cc. or less in persons with varying degrees of renal insufficiency. An alternate method of calculation involves extrapolation to zero time of the plot of log plasma  $I^{131}$  concentrations versus time, and division of the (hypothetical) value for plasma  $I^{131}$  so obtained into a quantity representing rate of renal excretion of  $I^{131}$ . (The latter value is derived by the procedure of Ketting, Power, Berkson and Haines, *Journal of Clinical Investigation*, in press.) The values for plasma clearance of  $I^{131}$  obtained by this method are essentially similar to those obtained by the conventional method. These preliminary observations indicate, insofar as the clearance of tracer amounts of  $I^{131}$  may be accepted as a valid measure of the clearance of the nonradioactive inorganic iodide of the plasma, that the clearance of the latter under the conditions of our observations is considerably greater than that of the vastly more abundant halogen, chloride.

**N-acylated and N-methylated glycyldehydroalanine and related compounds** VINCENT E. PRICE (by invitation) and JESSE P. GREENSTEIN. *National Cancer Institute, National Institute of Health, Bethesda, Maryland*. Chloroacetyl-glycyldehydroalanine, glycylglycyldehydroalanine, and N-methylglycyldehydroalanine, in which one hydrogen atom of the  $\alpha$ -amino group is substituted, and chloroacetyl-N-methylglycyldehydroalanine in which both hydrogen atoms of the amino group are substituted, were prepared and subjected to enzymatic hydrolysis at pH 8 in certain rat tissue digests. The first two compounds were hydrolyzed in all tissue digests at close to the same rate as glycyldehydroalanine and were presumably attacked by dehydropeptidase I. No significant increase in  $\alpha$ -amino nitrogen was noted in ammonium-free, kidney digests of chloroacetyl-glycyldehydroalanine after the dehydropeptide bond was nearly completely hydrolyzed. N-methylglycyldehydro-

alanine was hydrolyzed at a lower rate than glycyldehydroalanine, while chloroacetyl - N - methylglycyldehydroalanine was not hydrolyzed in any of the tissues studied. To serve therefore as a substrate for dehydropeptidase I, the compound need not possess a free, basic  $\alpha$  amino group, but it must evidently have an  $\alpha$  nitrogen atom to which at least one hydrogen is attached. Under certain conditions, reaction of chloroacetyldehydroalanine with methylamine or dimethylamine leads not only to replacement of the halogen but also to substitution at the double bond.

**Purification and properties of *E. coli* bacteriophage T<sub>6</sub>.** FRANK W. PUTNAM, LLOYD M. KOZLOFF (by invitation) and E. A. EVANS, JR. *Department of Biochemistry, University of Chicago*. Study of protein synthesis and nucleoprotein turnover in virus-infected organisms has been initiated using the *E. coli* bacteriophage system as a model. By differential centrifugation in the Sharples supercentrifuge and in the high speed angle centrifuge, bacteriophage T<sub>6</sub><sup>+</sup> parasitizing *E. coli* (Strain B) has been isolated in a yield exceeding 8 mg per liter of crude lysate. For phage purified from nutrient broth medium the infectivity (gm N per infectious unit) measured by the plaque count method averaged  $10^{-15.34}$ . Elemental and component analysis, including P partition of the purified phage has been made. Desoxyribonucleic acid (DNA) is the characteristic nucleic acid. Physical characterization of the purified phage is being undertaken. Factors stimulating or inhibiting virus reproduction in broth or in synthetic (lactate) medium have been studied by the one step growth curve technique. While common metabolic inhibitors such as cyanide, azide, etc. only partially repress growth or lysis, addition of a cationic detergent (1/10,000) toward the end of the latent period completely inhibits phage reproduction. Phage containing radioactive phosphorus (infectivity  $10^{-15.75}$ , activity 66 counts/microgram P) has been isolated from a broth lysate containing 0.46 microcuries P<sup>32</sup>/ml, and the specific activity of the P fractions measured. Preliminary experiments indicate that the specific activity of the DNA fractions of the phage and the host bacterium are similar. Further radioisotope studies of the mechanism of phage synthesis are in progress.

**Analytical procedure for determining individual tocopherols in a mixture of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols.** M. L. QUAYE (introduced by P. L. HARRIS) *Research Laboratories of Distillation Products, Inc. Rochester, New York*. A method for determining  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols in a mixture containing all three as well as  $\alpha$ -tocopherol is presented. It depends on the formation of their yellow nitroso derivatives. These are separated by a simple chromatographic step and measured photometrically. The nitrosotocopherols are formed by

treating  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols in alcoholic solution with nitrous acid. Alpha-tocopherol gives no colored product. They are red in alkaline solution but revert to yellow when extracted into petroleum ether. They are measured with a Beckman spectrophotometer at 410 m $\mu$  or with an Evelyn photometer, using filter 400. Under the conditions used  $\gamma$ - and  $\delta$ -nitrosotocopherols have the same extinction, while  $\beta$ -nitrosotocopherol has about one-third less. The nitrosotocopherols can be separated by a simple chromatographic step and evaluated. They form separate red bands on the column with the  $\gamma$ -derivative at the bottom, the  $\beta$ - in the middle, and the  $\delta$ - (two bands) at the top. These are eluted separately according to the flowing chromatogram technique. Residues of the eluates are dissolved in petroleum ether and their absorption measured as noted. Spectra of the eluates were found to be identical to those of control unchromatographed solutions of the nitrosotocopherols. Synthetic mixtures of all three non- $\alpha$ -tocopherols in the presence of  $\alpha$ -tocopherol have been separated and the components recovered quantitatively. The method has been applied to commercial vitamin E concentrates which contain all four tocopherols and to vegetable oils, following laboratory molecular distillation.

**Gas pressure regulation in flame photometry.** JOS M. QUASHNOCK (introduced by WM. S. McELLENOR) *Department of Physiological Chemistry, School of Medicine, University of Pittsburgh*. A Perkin Elmer flame photometer with a Rangelator 106 64 was studied to measure the degree of gas pressure regulation and the influence of any variation on the accuracy of the determinations. Gas pressure was measured for four hour periods while the photometer was in use. Maximum pressure change was 0.8 mm. water with a mean working pressure of 81.0 mm. The mean time elapsing between successive minimum and maximum pressures was 5.4 seconds. Measurements were then made on Na and K standards corresponding to normal sera diluted 1/500 for Na and 3/100 for K. The instrument was set at 100 with 10 p.p.m. Na or K standard. The various concentrations were determined at gas pressures of 80 mm. and 78 mm. water in rapid succession. This pressure change gave greatest differences in measurements of high concentrations. At a concentration corresponding to 8.5 meq/l. the results were 4.2% lower for the 78 mm. pressure than for 80 mm. Na measurements at 217.5 meq/l. were 2% apart. Variations in measurements of Na in the normal range of concentrations were less than 1%, while the differences for K were too small to be measurable. Since the maximum pressure change under operating conditions is 0.8 mm. water, the actual error will be less than half of the above. The gas pressure regulator studied is adequate and gives variations which do

not significantly influence the accuracy of the flame photometer for measurements of normal serum Na and K

**Role of platelets in coagulation of blood evidence of an inhibitor of the platelet factor** ARMAND J. QUICK *Department of Biochemistry, Marquette University School of Medicine, Milwaukee* It has recently been found that platelets do not supply thromboplastin, but an agent which activates the thromboplastin precursor (thromboplastinogen). When blood is collected under conditions in which the platelets are scrupulously preserved (i.e. at low temperature and with methylchlorosilane coated instruments and containers) and is centrifuged at high speed to remove the platelets, a plasma is obtained which clots very slowly and in which little prothrombin is converted to thrombin. When washed hemophilic platelets are added to such a plasma, a normal coagulation time and a significant increase in prothrombin consumption results. Likewise, the addition of this platelet free plasma to hemophilic blood normalizes the coagulation time and increases the prothrombin consumption. This is accomplished by supplying thromboplastinogen which hemophilic blood lacks. There is no evidence that it contains any inhibitory agent. Blood obtained from a patient 69 years old, who developed a hemophilia-like disease following a dermatitis, had a coagulation time of 35 minutes. When this blood was added to an equal volume of normal blood (coagulation time 6 minutes) the mixture coagulated in 30 minutes and only a trace of prothrombin was activated. These findings suggest that the patient's blood contained an inhibitor of the platelet factor and that therefore the conversion of thromboplastinogen to active thromboplastin was prevented. This type of anticoagulant is particularly significant clinically because it cannot be counteracted by transfusion with normal blood.

**Enzymatic formation and breakdown of pentose phosphate** E. RACKER (introduced by S. OCHOA) *Department of Bacteriology, New York University College of Medicine* The oxidative decarboxylation of phosphogluconic acid to a pentose phosphate and the fermentation of the latter to  $\text{CO}_2$  and water was previously shown to occur in yeast extracts by Warburg, Christian and Griese (*Biochem Z* 282: 157, 1935) and by Dickens (*Biochem J* 32: 1626, 1938). A triphosphopyridine nucleotide enzyme, oxidizing phosphogluconic acid, has now been found in bacterial extracts and has been separated by ammonium sulfate fractionation from the enzyme which oxidizes glucose 6-phosphate to phosphogluconic acid (Zwischenferment). In bacterial extracts an enzyme has been found which produces triosephosphate from ribose-5-phosphate. The activity of this enzyme has been measured

spectrophotometrically in the presence of an excess of triosephosphate isomerase and  $\alpha$ -glycerophosphate dehydrogenase. The latter enzyme converts the triosephosphate which is formed from ribose-5-phosphate, into  $\alpha$ -glycerophosphate in presence of reduced diphosphopyridine nucleotide (DPN). The oxidation of reduced DPN is followed by the disappearance of absorption at  $\lambda$  340 m $\mu$ . The ribose 5-phosphate splitting enzyme from bacterial extracts has been partially purified. In the same extracts an enzyme is present which oxidizes reduced DPN in the presence of glyceraldehyde. Glyceraldehyde and triosephosphate can condense to give a pentose phosphate. This reaction is catalyzed by crystalline aldolase prepared from rabbit muscle. The barium salt of the pentose phosphate has been isolated. It is not ribose 5-phosphate and no evidence is available that it can be converted into ribose 5-phosphate.

**Enzymatic inactivation of serum vasoconstrictor** MURIEL M. RAIPORT (by invitation) ARDA ALDEN GRIFFIN and IRVINE H. PAGE *Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio* It is well established that perfusion of lung with serum or defibrinated blood destroys their vasoconstrictor activity. The mechanism of this destruction is probably enzymatic since a protein fraction from lung extracts inactivates highly purified preparations of serum vasoconstrictor.

**Rate of protein formation in the livers of partially hepatectomized rats** D. RITTENBERG, LOUISE L. SPROUL (by invitation) and DAVID SHIMM *Department of Biochemistry and Pathology, College of Physicians and Surgeons, Columbia University, New York* While in a non growing organism the rate of the synthetic reactions is identical with the rate of the degradative reactions, this cannot be the case in a growing organism. Growth results because the rate of synthesis exceeds that of degradation. Previous work from this laboratory has given values for the rate of formation of the proteins of the adult rat liver. We have now investigated the rate of protein formation in the livers of partially hepatectomized rats. Two days, after about 50% of the liver had been removed, the rats were given a standardized amount of glycine labeled with  $\text{N}^{15}$ . The rate of protein formation was measured by determining the rate of incorporation of  $\text{N}^{15}$  into the liver proteins. The data indicate that the rate of protein formation in the livers of these rats is not appreciably faster than in the livers of the normal animal. Growth in this instance is not the result of an acceleration of the synthetic mechanisms but rather appears to result from the inhibition of the degradative reactions. The implications of this concept of growth will be briefly discussed.

**Determination of arginase activity in tissue**

**homogenates application to epidermal carcinogenesis in mice** EUGENE ROBERTS (introduced by MICHAEL SOMOGYI) *Barnard Free Skin and Cancer Hospital, St. Louis, Mo* Previously described methods for the determination of arginase activity have been modified to give satisfactory results with distilled water homogenates of mouse liver, kidney, epidermis, and squamous cell carcinoma. An aliquot of a 2% homogenate of the tissue was incubated with an equal volume of 2%  $MnCl_2$  for 5 to 6 hours with frequent shaking. Maximal activation was achieved within 4 hours with liver, epidermis, and tumor, and was maintained through at least 6 hours of incubation. No activation of kidney homogenate was obtained with this procedure, and almost complete inactivation of the enzyme in this tissue occurred within 6 hours. The activities of suitable quantities of the homogenates before and after activation were measured in the presence of excess  $MnCl_2$  by estimation of the urea formed during incubation for 10 minutes at  $38^\circ C$  with a concentrated arginine solution. The reaction was of zero order and the activity for each tissue was proportional to the quantity of tissue employed. Arginase activity values expressed in micrograms of urea liberated per mg of tissue nitrogen (a) before activation and (b) after activation are given below for 3 sets of tissues consisting of 10 samples each studied in epidermal carcinogenesis induced in mice by methylcholanthrene: normal epidermis, (a) 146 (61-220) and (b) 1171 (842-1659), hyperplastic epidermis, (a) 1221 (897-1682) and (b) 3074 (2,240-4380), squamous cell carcinoma, (a) 1992 (861-2390) and (b) 13,378 (5,950-18,700). These results show that a highly significant increase in arginase activity occurs in epidermal carcinogenesis.

**Biochemical characterization of lymphoid tissue proteins** SIDNEY ROBERTS (by invitation) and ABRAHAM WHITE *Department of Physiological Chemistry, Yale University, New Haven* Mildly alkaline (pH 7.6) extracts of lymphoid tissue obtained from rabbit, mouse lymphosarcoma transplants, and calf thymus have been found to be qualitatively similar in the Tiselius apparatus. Six to eight components are evident, the quantity of each varying with species and with efficiency of extraction. Approximately 90% of the total protein present is distributed among four fractions, one of which represents more than half of the total protein in the extracts. Fractionation studies have been conducted with alkaline extracts of calf thymus. Treatment with cold ethanol yields fractions insoluble at  $10^\circ C$ ,  $20^\circ C$ ,  $30^\circ C$ , and  $40^\circ C$  ethanol concentrations respectively. Electrophoretic examination of these fractions suggests that a partial separation of thymus proteins has been achieved. Preliminary observations have been made on some of the chemical and physiological properties of these thymus

proteins. Daily injection of the initial extract of calf thymus into the rat resulted in hypertrophy of the spleen and thymus in one to two weeks. An apparent inhibition of the normal lymphopenic effect of foreign protein injection in the rat has also been observed, this activity appeared to be characteristic chiefly of the precipitate obtained with 40% ethanol.

**A fluorimetric assay for N<sup>1</sup>-methyl-6-pyridone-3-carboxylamide** FRED ROSEN, (by invitation) W. A. PERLZWEIG, and PHILIP HANDLER *Department of Biochemistry, Duke University School of Medicine, Durham, N. C.* Incubation of N<sup>1</sup>-methyl-6-pyridone-3-carboxylamide (I) with acetone and KOH yields a fluorescent derivative. Traces of water inhibit the reaction. Fluorescence disappears if the final solution is acidified and may be restored by adding alkali. N<sup>1</sup>-methylnicotinamide does not yield a fluorescent compound under these circumstances. The following procedure, while not entirely satisfactory, has been employed for urine analysis. To 10 ml urine, adjusted to pH 9.6, 3.0 ml saturated lead subacetate are added. After centrifugation the supernate is brought to pH 6 and evaporated in a porcelain dish. The residue is transferred to a test tube with 0.5 ml water and washed in with about 9.5 ml acetone. After adding 2 gm anhydrous  $Na_2SO_4$  and centrifuging, the supernate is evaporated and the residue extracted with a total of 8 ml acetone. A suitable aliquot (0.2-1.0 ml) is diluted to 1.5 ml with acetone and 0.06 ml 12 N KOH added. The tube is covered with parafilm and shaken for 3 hours at  $20^\circ$ . The solution is diluted to 10 ml with water and read in a suitable, sensitive fluorometer using thiamine assay filters. A blank is prepared by adding 0.1 ml water to a similar tube before the incubation period. The average 24 hour excretion of I by 6 normal adults was 8.4 mg (5.0-13.2). After oral administration of 200 mg nicotinamide, 150 mg I was excreted within 24 hours and proportionally more after larger doses. Somewhat less appeared after corresponding doses of niacin. The errors of the urine extraction procedure are such that these values are probably somewhat low. Nevertheless, they establish I as the major end product of nicotinamide metabolism.

**The distribution of rhodanese** OTTO ROSENTHAL *Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia* Rhodanese catalyzes the reaction  $HSCN + Na_2S O_3 \rightarrow NaSCN + NaHSO_3$ . Mendel, Rudnev, and Brown reported that the enzyme was absent in transplanted murine sarcomas and normal murine jejunal mucosa, tissues displaying high aerobic glycolysis. These authors suggested that the aerobic glycolysis was due to an inhibition of the Pasteur reaction by small amounts of HCN formed in the tissue metabolism and accumulating in the absence of rhodanese. We have determined

the rhodanese activity of  $\text{H}_2\text{O}$  homogenates of diverse normal and neoplastic tissues from different animal species. The activity was expressed in terms of  $\mu\text{M}$  thiocyanate/min/gm dry weight of tissue measured at  $20^\circ\text{C}$  in phosphate buffer of pH 7.3, the substrate concentration being 0.05 M. The values obtained ranged from 2 to 5 in murine sarcomas and jejunal mucosa, rabbit spleen and pale skeletal muscle up to 600 in rat liver. There were pronounced species differences in the activity of homologous tissues. While our data confirm the experimental findings of the previous workers they also demonstrate that rhodanese deficiency occurs in tissues with a normal aerobic metabolism. The biological significance of rhodanese remains to be ascertained. Studies on the kinetics of the rhodanese reaction revealed apparent dissociation constants of the enzyme substrate complex of  $5.5 \times 10^{-3}$  and  $3.8 \times 10^{-3}$  for  $\text{S}_2\text{O}_3^{2-}$  and  $\text{HCN}$  respectively. The affinity of  $\text{HCN}$  to rhodanese is thus smaller than to the respiration enzyme. Under our conditions of assay 0.05 M sulfite produced a 50% reduction of the rate indicating reversibility of the reaction.

**Early changes in the seminal vesicles of the castrate rat following administration of testosterone.** G. G. RUDOLPH (by invitation) and L. T. SAMUELS, *Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah*. Fifteen and twenty hours after the subcutaneous injection of one mg of testosterone propionate in the castrate rat there is a significant increase in the weight of the seminal vesicles. The glands have an increased water content twenty hours after injection and at this time there is an increase in the

tracellular water as measured by chloride space. The oxygen uptake of the seminal vesicles increases as early as 10 hours after the injection of testosterone propionate, and this level is the same as the oxygen consumption of the glands fifteen hours and twenty hours after injection. The oxygen uptake at these times is the same as that of the seminal vesicle from a normal mature rat. When sodium succinate is used as substrate, the oxygen uptake of the seminal vesicles from castrate animals that had been injected for ten hours was increased over the oxygen uptake of the glands with glucose as substrate, while the oxygen uptake of the glands from uninjected castrate animals was only slightly increased. The changes in the glands which bring about this increased oxygen uptake will be discussed.

**Oxidation of members of the Krebs cycle by liver and kidney. Inhibition by trans-aconitate.** MURRAY SAFFRAN and J. L. PRADO (both introduced by K. A. C. ELLIOTT), *Department of Biochemistry, McGill University*. The addition of members of the tricarboxylic acid cycle to kidney cortex slices results in considerable acid disap-

pearance, increased oxygen uptake, and R. Q. changes, indicating complete oxidation of these substances. This is not the case with slices of other tissues (Elliott, K. A. C., *Physiol. Rev.*, 21, 267 (1941)). Similar behaviour has been found with isotonic suspensions of liver and kidney cortex. The following observations were made during the course of an investigation of this difference. Low concentrations of trans-aconitate, an inhibitor of aconitase, depress the respiration of kidney slices in complete Ringer bicarbonate or phosphate, but scarcely affect liver slices. In calcium free medium, however, both are affected. The inhibitory effect is approximately proportional to the logarithm of the trans-aconitate concentration. Decreased respiration is found when no substrate is added, or when an excess of fumarate or malate is present, but the inhibition is overcome by the addition of succinate, citrate or cis-aconitate. Presumably the intense activity of the succinate oxidizing system is not affected, and in increased concentrations, cis-aconitate and citrate overcome the competitive inhibition by trans-aconitate. In the absence of calcium, high concentrations of malate cause appreciable acid disappearance with liver slices, though the rate of oxygen uptake is actually depressed. It is tentatively concluded that both liver and kidney possess the enzymes necessary for the complete utilization of Krebs cycle acids, but that permeability factors prevent the complete oxidation of these substances by slices of tissues other than kidney.

**The effects of thiamine deficiency in rats forced to maintain caloric balance.** LEO T. SAMUELS, A. A. ANDERSON (by invitation), E. CHAPMAN (by invitation) and D. MACKEY (by invitation), *Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah*. To prevent the development of multiple deficiencies on thiamine deficient diets due to the loss of appetite, rats were fed diets of constant composition by stomach tube. Both controls and deficient groups received sufficient calories to cause an increase in total weight. After 17-20 days gastric atony developed in the deficient rats. Within three days after its appearance, all these rats would be dead from gastric distention unless feeding was stopped. Consequently the rats were killed at this time and their tissues analyzed. There was no evidence of inability to form fat, both the deficient and control rats having shown a slight gain in liver fat and a marked increase in carcass fat. The nitrogen content of the deficient rats was higher and the glycogen lower than their respective controls. The kidneys of the deficient rats were 30% larger than those of the controls. Pyruvate levels in deficient rats and pyruvate oxidation were also studied.

**Blood and "Tissue" protein changes in dogs on protein-deficient diets with and without sup-**



**plementation** GEORGE S SAMUELSEN (by invitation), GRACE E GRIFFIN (by invitation), LOIS E GRIFFITH (by invitation), SAM SEIFTER and EDWARD MUNTWYLER *Department of Biochemistry, Long Island College of Medicine, Brooklyn* Determinations were made of the plasma volume, hematocrit, hemoglobin, total blood and plasma proteins, plasma albumin, and nitrogen balance of groups of dogs maintained respectively for three weeks on the following diets (1) control synthetic diet containing 20% casein, (2) protein free, (3) protein free, choline-free, (4) protein-free plus methionine, (5) protein free, potassium-free, and (6) a gelatin diet Chemical studies were also made during two subsequent periods of supplementation with commercial hydrolysates of casein and lactalbumin During depletion, the urinary nitrogen was derived mainly from sources outside of the circulation except in the case of the gelatin diet Whereas the relative amounts of blood nitrogen and "tissue" nitrogen (nitrogen balance minus blood nitrogen) lost were reasonably constant on a given diet, their proportion varied markedly with the type of diet Elamm's ratio of 1.30 for plasma albumin loss to total body protein loss did not hold for all diets, the protein free plus methionine diet resulted in a ratio of 1.50, the gelatin 1.13 With nitrogen supplementation following depletion, the tissues claimed a priority for the retained nitrogen The return to normal of circulating plasma protein did not necessarily indicate that "tissue" proteins were restored or *vice versa* Methionine, and choline to a lesser extent, had a nitrogen sparing effect, the methionine sparing blood protein especially Omission of potassium from the protein free diet did not further alter nitrogen balance Poor regeneration of blood proteins was observed after supplementation of both the potassium free and gelatin diets

**Amino acid excretion as influenced by dietary proteins of different biological value** H E SAUBERLICH (by invitation) and C A BAUMANN *Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis* Mice fed diets devoid of an essential amino acid are known to excrete very high percentages of all the ingested amino acids into the urine This observation has been extended to another species, the rat, and studies were also made with mice to determine whether proteins of greater biological value than casein might not depress amino acid excretion Rats fed diets deficient in the essential amino acids, methionine or tryptophane, excreted approximately twice the amounts of amino acids in the urine as those excreted by rats fed an adequate protein On diets devoid of methionine or tryptophane, rats excreted 2.3% and 3.9% of the ingested tyrosine in the urine, while rats fed casein excreted only 0.7% of the ingested tyrosine in the urine

Restriction in food intake further reduced the excretion of all amino acids Mice fed diets of a high biological value excreted less amino acids in the urine than mice fed proteins of ordinary or poor biological value, e.g. mice fed diets containing 8% of arachin, casein, fibrin, egg albumin, or lactalbumin excreted 5.0%, 4.4%, 3.3%, 1.0%, and 0.9% of the ingested histidine in the urine, respectively The mean excretion for the seventeen amino acids determined by microbiological procedures was 4.7% for arachin, 3.4% for casein, 2.7% for fibrin, 1.5% for egg albumin, and 1.0% for lactalbumin

**Some properties of a transsulfurase responsible for conversion of cyanide to thiocyanate** J P SAUNDERS and W A HIMWICH (introduced by BERNARD J JANDORF) *Toxicology Section, Medical Division, Army Chemical Center, Maryland* The effect of separately varying time, temperature, pH and substrate (cyanide and thiosulfate) concentration was studied on the enzyme catalyzing the conversion of -CN to -CNS Enzyme activity increased sharply with time of incubation until 40 minutes, from which point there was no appreciable increase with time A sharp activity optimum was observed at 37°C Different pH optima were shown depending upon the species from which tissue homogenates were prepared Brain and liver tissues from dog, rat and rhesus monkey showed an optimum at pH 7.3-7.5, while tissues from rabbit gave a slightly higher optimum at pH 7.7-7.9 The pH activity curve for rabbit tissues showed a much broader peak near the optimum than tissues from the other species examined Maximum activity was observed when the substrate concentration was such that molar thiosulfate concentration was three times that of the cyanide present No appreciable loss of activity was observed in homogenates stored for as long as 96 hours at 5°C, or in tissues kept for four hours at room temperature The enzyme is inhibited by a number of compounds including S containing compounds and -CN If CN is added to the tissue homogenate before addition of thiosulfate, almost complete inhibition is effected, whereas if the same amount of -CN is added after the addition of thiosulfate, normal activity is obtained This suggests the possibility that a loose thiosulfate enzyme combination is formed, which gives up sulfur in the presence of cyanide to form thiocyanate

**Inhibition of peptic activity by hydrazine** OTTO SCHALLS, REGINA M ROUX (by invitation), and ANNE M SUTTON (by invitation) *Chemical Research Laboratory of the Alton Ochsner Medical Foundation and Department of Biochemistry, Tulane University, New Orleans, La* Josephson and Euler (*Ztschr. physiol. Chem.* 162: 85, 1927) observed inhibition of pepsin by phenylhydrazine, cyanide and bisulfite and concluded that this enzyme contains a group of aldehyde character

Rona (*Biochem Ztschr* 109 279, 1920) found no inhibition of pepsin and trypsin by aldehyde reagents. Experiments were carried out in this laboratory in which the disappearance of the turbidity of an egg-white suspension in presence of pepsin, trypsin and papain was followed photoelectrically, using a modification of the method of Riggs and Stadie (*J Biol Chem* 150 163, 1943). A variety of aldehyde reagents decreased the rate of enzymatic hydrolysis of this substrate. Hydrazine was the most effective inhibitor for pepsin. Peptic hydrolysis followed a monomolecular course during the interval from 5-10 min and the reaction constant  $k$  was a measure of pepsin activity. It was found that (1) Two millimol hydrazine per liter decreased  $k$  to one-half its original value. (2) There was a linear relationship between  $k$  and the logarithm of the hydrazine concentration. (3) Both in the presence and absence of hydrazine there was a linear relationship between  $k$  and the reciprocal of the initial substrate concentration. (4) Graphical interpretation of experimental data indicated that the inhibition was of the non competitive type. It is concluded that the pepsin molecule contains one or more groups which react with hydrazine and which are probably of aldehydic or closely related nature. The non competitive type of inhibition suggests that these "activating" groups do not combine with the substrate during the proteolytic reaction.

**Purification of phosphoglucomutase with "carbitol" acetate.** MAX SCHLIMOWITZ (by invitation) and DAVID M. GREENBERG, *Division of Biochemistry, University of California Medical School, Berkeley*. The preparation of phosphoglucomutase from rabbit muscle with greater activity than could be gotten by previously described methods has been achieved by fractionation with "carbitol" acetate (diethylene glycol monoethylether acetate) used in conjunction with selective heat denaturation and salting out techniques. Phosphoglucomutase isolated by the method of Colowick and Sutherland (*J Biol Chem*, 144 423 (1942)), which involves precipitation of the enzyme from an aqueous extract of muscle with ammonium sulfate (0.4-0.6 sat.) followed by selective heat denaturation (30 min at 50-52° and pH 5.15), has yielded preparations of phosphoglucomutase with activities ranging up to 3,300 units per mg protein. Phosphoglucomutase preparations with activities of 6,000 units per mg protein could readily be obtained from aqueous muscle extract by fractionating with "carbitol" acetate (40-60% by volume). Selective heat denaturation raised the activities to around 10,000 units per mg protein, and salting out with ammonium sulfate (0.5-0.7 sat.) further increased the activity values to 13,500-17,200 units per mg protein. The phosphoglucomutase prepared in this manner was free from phosphorylase and

hexoseisomerase activity. The enzyme unit mentioned above is defined as the  $\gamma$  of phosphorus of glucose-1-phosphate converted to glucose 6-phosphate phosphorus by 1 mg of enzyme protein in 30 min in a medium containing glucose-1-phosphate (0.162 M),  $Mn^{++}$  (1.25 mM) and  $CN^-$  (3mM) at pH 7.1.

**Ribopolynucleotide fractions formed during the enzymatic hydrolysis of yeast ribonucleic acid.** GERHARD SCHMIDT, RICARDO CUBILES (by invitation) and S. J. THANNHAUSER, *Boston Dispensary, Tufts College Medical School, Boston*. It was demonstrated (Cold Spring Harbor Symp., vol. 12, 1947, *J Biol Chem*, 170 761, 1947) that the inorganic P released by prostatic phosphatase during a relatively short incubation period from ribonuclease-treated nucleic acid originates exclusively from pyrimidine nucleotide groups. It was concluded that the action of ribonuclease involves specifically, or at least preferentially, the pyrimidine nucleotide groups of the RNA molecule and that the enzymatic digest contains polynucleotides in which the proportion of purine to pyrimidine nucleotide groups is higher than 1:1.

The isolation of two of these polynucleotide fractions in the form of water-insoluble Ba salts has now been carried out. The action of ribonuclease alone leads to the formation of a polynucleotide fraction in which the ratio purine nucleotide:pyrimidine nucleotide is approximately 2:1. This fraction seems to correspond to the split product described by Loring et al. (*J Biol Chem*, 169 601, 1947) who also found that it had a high purine:pyrimidine ratio. A second polynucleotide fraction was obtained after digesting the first fraction with large amounts of prostatic phosphatase. This fraction contained exclusively purine nucleotides. The polynucleotide nature of the free acids was established 1) by their low solubility in acids, 2) by their relatively very slow hydrolysis by acid phosphatase, 3) by the results of titration. These properties distinguish the polynucleotide fractions from guanylic acid which likewise forms a water-insoluble Ba salt or from mixtures of mononucleotides.

**Substances isolated following the incubation of dehydroisoandrosterone, androsterone and etiocholanolone with liver tissue.** JOHN J. SCHNIDLER (by invitation) and HAROLD L. MASON, *Division of Biochemistry, Mayo Clinic, Rochester, Minnesota*. Dehydroisoandrosterone, androsterone and etiocholanolone were incubated as the sodium salts of their hemisuccinates with surviving rabbit liver slices for periods of three and six hours under aerobic conditions. The proteins then were precipitated with acid acetone and thoroughly washed with fresh acetone. After removal of the acetone in a vacuum the aqueous residue was made alkaline and extracted with ethylene dichloride in order to

obtain the free steroids. Further extraction after acidification of the aqueous phase gave a conjugated steroid fraction. Separation into ketonic and nonketonic fractions was accomplished with Girard's reagent T and isolation of compounds by use of chromatographic columns. Dehydroandrosterone was converted largely to  $\Delta^5$  androstene-3( $\beta$ ),17( $\alpha$ )-diol and in small yield to  $\Delta^5$  androstene-3( $\beta$ ),16( $\beta$ ),17( $\alpha$ )-triol. In order to study the role of the diol as a possible intermediate in the conversion of dehydroandrosterone to the triol,  $\Delta^5$  androstene-3( $\beta$ ),17( $\alpha$ ) diol dihemisuccinate was incubated under the conditions indicated. Only the diol and a small amount of dehydroandrosterone were recovered. Compounds formed during the incubation of androsterone included androstane 3,17 dione, androstane 3( $\alpha$ ),17( $\alpha$ )-diol and several as yet unidentified compounds, two of which may be isoandrosterone and androstane-3( $\alpha$ ),17( $\beta$ ) diol. Etiocholanolone was converted to etiocholane-3/17-dione, etiocholane 3( $\alpha$ ),17( $\alpha$ )-diol and etiocholane 3( $\alpha$ ),17( $\beta$ ) diol. The significance of these results in relation to ketosteroid metabolism in general will be discussed.

**Absence of a sparing action of tryptophane on nicotinamide requirements of the fly, *Drosophila melanogaster*** JACK SCHULTZ and GEORGE T. RUDIN (introduced by GERRIT TOENIES) *Lankenau Hospital Research Inst., and Inst. for Cancer Research, Philadelphia 30, Pa.* A chemically defined medium for the growth of the fly *Drosophila melanogaster*, under bacteriologically sterile conditions, has been developed (Schultz, St. Lawrence and Newmyer, *Anat. Rec.* 96 4/44 (1947)). This furnishes a convenient system in which effects of components of the medium on growth can be distinguished from possible effects of symbiotic microorganisms. The amino acids essential for the rat are also essential for *Drosophila*, as are the vitamins of the B complex. In the work to be reported, the growth on concentrations of tryptophane from 0.06 to 0.23 mg. per ml., at levels of nicotinamide varying from 2.5 to 40 gamma per ml. medium, has been studied. Growth was measured in terms of larval length, pupation time, and weight of the adult fly. The wild type *Drosophila* and a vermilion eyed mutant, which lacks the ommatin pigment of which tryptophane is a precursor, were used. In both types the nicotinamide requirements for optimum growth were found in general to increase with the tryptophane supplied. This is in apparent contradiction to the well known findings in mammals concerning the sparing action of tryptophane on nicotinamide. The contradiction is resolved by the recent finding of Ellinger and Abdel Kader (*Nature* 160 675 (1947)) that the major responsibility for the nicotinamide tryptophane interrelation rests upon the microorganisms of the gut. In the vermilion mutant, the

genetic disability to use tryptophane for pigment formation lowered the tryptophane requirement for optimum growth.

**Influence of the presence of a sterile abscess on the detoxication of brombenzene as mercapturic acid** JULIUS SCHULTZ (introduced by HARRY M. VARS) *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia*. The amount of mercapturic acid-S excreted by the rabbit following the oral administration of monobrombenzene was found to be increased in the presence of a sterile abscess. These increments were observed in both fed and fasted rabbits. In the fed rabbit receiving a single 1.5 gm. dose of brombenzene, the mercapturic acid S was 43 and 51 mg. without a sterile abscess and 61 mg. with. In another rabbit 1.5 gm. of brombenzene on 2 successive days yielded 86 mg. of mercapturic acid-S. In the presence of a sterile abscess the excretion was 105 mg. mercapturic acid-S. During a 2 day fast the rabbits receiving 2 successive doses of brombenzene excreted 36 and 34 mg. of mercapturic acid S. These same animals in the presence of a sterile abscess produced 42 and 54 mg. respectively. On the basis of sulfur partitions observed with these data, it can be shown that following a sterile abscess there was an increase in neutral sulfur. It appears that this neutral sulfur may be utilized by the animal in forming mercapturic acid. These data indicate that a specific metabolic reaction can utilize the tissue breakdown products resulting from the formation of a sterile abscess. The significance of these findings in relation to the protective action of a sterile abscess against chloroform poisoning will be discussed.

**Studies on folic acid conjugase in blood** B. S. SCHWEIGERT (introduced by P. B. PEARSON) *Dept. of Biochemistry and Nutrition, 1 & M College of Texas, College Station*. In the course of investigations on the influence of the dietary intake of pteroylglutamic acid (folic acid) on the amount present in blood before and after enzymatic treatment, the occurrence of folic acid conjugase in blood has been demonstrated. Microbiological analyses on samples of whole blood or plasma (turkey) incubated at pH 7.0 for 16-18 hours were 5-20 times higher than analyses made on similar samples prior to incubation. Separate studies showed that greater liberation of the vitamin occurred when the blood was incubated at pH 7.0 or 8.0 than at pH 4.5 or 6.0. A further increase in the amount liberated was obtained when the samples were incubated with a chick pancreas enzyme preparation (pH 7.0). Preliminary evidence indicates that the concentration of folic acid in blood determined either before or after enzymatic treatment (chick pancreas enzyme) is influenced by the amount of the vitamin ingested by the turkey.

Turkeys maintained on a purified ration deficient in pteroylglutamic acid showed poor feathering, a slow rate of growth and a low hemitocrit as well as a low concentration of pteroylglutamic acid in the blood

**Purification of alpha-amylase from barley malt extracts** SIGMUND SCHWIMMER *Enzyme Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA, Albany, California* The Maltose Equivalent of purified enzyme preparations has been raised from 350,000 as previously reported (Cereal Chem., **24**, 315 (1947)), to 130,000 units of alpha amylase per mg of protein. The new preparations were made from a commercially obtained concentrated barley malt extract instead of whole malt. This extract contains smaller amounts of the less soluble protein impurities which are present in usual grain extracts. Adsorption of the enzyme on bentonite was no longer required and purification was achieved merely by a series of ammonium sulfate fractionations of the heated extract. The less soluble protein impurities present at later stages of purification include an iron-rich globulin which apparently forms a solid solution with the enzyme protein on precipitation. After each fractionation step, some of the less soluble impurity can be removed by dialysis against saturated calcium sulphate solution. The more soluble protein impurities remain in the supernatant of the precipitated enzyme solution. Preliminary ultracentrifuge measurements on a preparation so purified indicate that the bulk of the protein present sediments as one component.

**Bacteriological characteristics of mandelamine**

J. V. SCUDI and CHARLES J. DUCA (by invitation) *Nepera Chemical Co. Inc., Yonkers, N. Y.* Mandelamine (methenamine mandelate) was compared with Streptomycin and with Sulfathiazole. Each drug was dissolved in sterile, buffered urine using a serial two fold dilution technique, and 17 strains of bacteria commonly associated with urinary tract infections were then challenged with each of the three drugs. Bacteriostatic and bactericidal studies showed that Mandelamine compares very favorably with Streptomycin and with Sulfathiazole in activity. Minimal bacteriostatic concentrations of Mandelamine were generally the same as its bactericidal concentrations. Lowering the urinary pH from 6.5 to 5.5 increased the activity of Mandelamine. Resistance to Streptomycin and Sulfathiazole appeared rapidly and to a marked degree. Organisms rendered resistant to these drugs at one pH remained resistant at higher pH levels. Further, organisms rendered resistant to these drugs remained fully susceptible to Mandelamine. In sharp contrast with the results obtained with Streptomycin and Sulfathiazole, it was impossible to elicit any direct evidence of

resistance to Mandelamine. The clinical implications of these findings are discussed.

**Pharmacological characteristics of neohetramine**, a new antihistaminic drug JOHN V. SCUDI, JOHN F. REINHARD (by invitation) and N. B. DREYER (by invitation) *Nepera Chemical Co., Inc., Yonkers, N. Y.* and the *Department of Pharmacology, University of Vermont* Neohetramine, 2 - (N - dimethylaminooctyl - N - p - methoxybenzyl) aminopyrimidine monohydrochloride, is relatively non-toxic and is efficient both in counteracting the effects of histamine and in preventing anaphylactic shock. The acute toxicity of Neohetramine in mice is about one half that of other antihistamines. Weanling rats receiving as much as 200 mg/kg of Neohetramine daily for 3 months grew at a normal rate, presented no abnormalities in blood morphology and developed no organ pathology. Neohetramine showed marked activity against the broncholar, capillary, local vasodilator, smooth muscle and vasodepressor actions of histamine, but the number of molecules of histamine antagonized by 1 molecule of Neohetramine varied from 0.001 to 9 depending on the species of animal and test procedure. Neohetramine protected against anaphylactic shock in actively and passively sensitized guinea pigs but the effective dosage was 5 to 20 times that required for comparable protection against histamine shock.

The general pharmacologic actions of Neohetramine are as follows: on smooth muscle, low concentrations are depressant, large concentrations induce contraction. In the eye, it produces a transient irritation accompanied by local anesthesia comparable to that obtained with procaine. It does not alter sympathetic responses, nor does it potentiate the action of epinephrine. Neohetramine enhances the intestinal response to vagal stimulation but it is also weakly parasympatholytic, causing slight depression of salivary secretion induced by stimulation of the chorda tympani nerve.

**Purification of prothrombin and thrombin** WALTER H. SELGERS and ARNOLD G. WARE (by invitation) *Department of Physiology, Wayne University, College of Medicine* The purification procedures described for obtaining highly active prothrombin (Aich Biochem **6**, 85, 1945) have been subjected to extensive study. The former work has been repeated approximately 200 times with various modifications and it is believed that approximately the methods previously described give the highest purification thus far achieved. Thrombin of purity equivalent to that described by Seegers and McGinty (J. Biol. Chem. **146**, 511, 1942) has also been obtained. Products of this kind were examined for us in the ultracentrifuge by Gerson Kegeles and J. W. Williams of the University of Wisconsin. Both proteins were found to be polydisperse. The prothrombin molecule (ap-

proximately 140,000) is approximately twice the size of the thrombin molecule (approximately 77,000). In recent work purified prothrombin has been obtained in stable form. This is accomplished by the simple expedient of storing the  $Mg(OH)$  eluate, described on page 88, *Arch. Biochem.* 6, 1945, at 5°C overnight instead of storing it at room temperature. This maneuver precludes the appearance of small amounts of thrombin in the final product. Small amounts of thrombin destroy prothrombin. It is estimated that the prothrombin product contains less than 1%  $\gamma$  globulin as impurity. This  $\gamma$  globulin activity can be destroyed by heating the prothrombin at 53°C for 2 hours.

**A quantitative method for a serum polysaccharide present in cases of tuberculosis and carcinoma.** F. B. SLIBERT, M. L. PFAFF (by invitation), and M. V. SLIBERT (by invitation). *Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.* A simple method useful for routine clinical analyses, involving the reaction between a carbohydrate complex in the serum and tryptophane in the presence of perchloric acid, has been utilized to detect the presence of a substance which appears in the sera of such diseases as tuberculosis and carcinoma of several types. The nature of this substance has been considered at length and it was finally suggested, largely on spectrophotometric data, that it might consist of a fructose compound or the nucleic acids and their hydrolysis products or derivatives. Very little, if any, dialyzes away from normal serum whereas a considerable proportion diffuses from the pathological sera, and the presence of nucleic acid could not be established in these diffusates. Further work is needed to establish its identity.

A statistically significant increase over the normal value was found even in cases of minimal active tuberculosis and as the disease progressed to moderately and far advanced stages, this substance progressively increased, in some cases to more than 200 per cent. The mean value for minimal cases which have become of questionable clinical significance is the same as for normals but that for the moderately advanced cases in this category is increased.

**Concentration changes of certain vitamins and enzymes in livers of rats on protein free diet.** SAM SEIFTER, DAVID M. HARNES (by invitation), B. FELDMAN (by invitation), LEONARD RUBIN (by invitation) and EDWARD MUNTZILER. *Department of Biochemistry, Long Island College of Medicine, Brooklyn.* The present report initiates an investigation into the possible relationships existing among enzyme activities, coenzyme concentrations, and protein levels of livers of animals on restricted protein intake. The effect of protein depletion was determined on (1) an enzyme having no known vitamin coenzyme (ar-

ginase), (2) an enzyme having a riboflavin coenzyme (d-amino acid oxidase), and (3) nicotinic acid and riboflavin, which are active in numerous enzyme systems. Later studies will deal with the coenzyme-enzyme-protein relationships. Three groups of Wistar albino male rats between 16 and 25 weeks of age were placed on a protein free diet, adequate in all other respects, for periods of one, two, and three weeks respectively. Pair fed controls were maintained concurrently on an adequate diet containing 20% casein. At the end of the feeding periods the animals were sacrificed without anesthesia and the livers analyzed for water, fat, nitrogen, riboflavin and nicotinic acid (microbiologically), d-amino acid oxidase (by pyruvate formation from alanine), and arginase (by urea formation from arginine).

Animals on the protein-free diet showed an increase in liver fat and a decrease in liver nitrogen as compared with the pair fed controls, and the changes were proportional to the duration of the depletion period. The livers of the depleted animals also showed a decrease in nicotinic acid, riboflavin, d-amino acid oxidase, and arginase as compared with the controls, all changes were proportional to the decrease in hepatic nitrogen.

**Lipotropic activity and toxicity of methoxamine (oxy methionine).** C. BOYD SHAFFER and FRANCES H. CRITCHFIELD (introduced by LEONARD H. CRETCHER). *Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh, Pennsylvania.* Four groups of 10 rats each were fed a lipogenic diet for a period of 21 days. The animals initially weighed between 130-180 gms, with equal numbers of males and females per group. The basal diet consisted of lard 40, casein 5, sucrose 46, salts 4, and cellulose 5. Purified vitamins were supplied in tablet form. In addition, the following supplements were given daily by stomach tube in water solution: Group II, 50 mg methoxamine; Group III, 50 mg methionine; Group IV, 50 mg methoxamine plus 50 mg methionine. Group I served as controls. All animals in Group II died between the 12th and 20th day, and are therefore excluded from further comparison. Those in the other groups lost weight rapidly, but all survived the experimental period. Liver and kidneys were weighed at sacrifice, and liver lipid (fatty acids plus cholesterol) determined by a micro-oxidative method. This and other pertinent data are tabulated below.

|                                  | Group I | Group III | Group IV |
|----------------------------------|---------|-----------|----------|
| Food intake gm./rat/day          | 4.16    | 4.11      | 4.24     |
| Mean wt. loss (% of initial wt.) | 16.60   | 13.20     | 34.40*   |
| Liver wt. (% of body wt.)        | 6.19    | 6.33      | 5.13     |
| Kidney wt. (% of body wt.)       | 0.87    | 0.92      | 1.05*    |
| Liver lipid (% wet organ wt.)    | 19.90   | 14.90*    | 7.02†    |

\* Statistically significantly different from Group I.

† Statistically significantly different from Groups I and III.

From this it is concluded that methoximine exerted a definite lipotropic effect, in addition to an inherent toxic action.

**The *in vitro* synthesis of heme from glycine by the nucleated red blood cell** DAVID SHLIM, IRVING M. LONDON (by invitation) and D. RIRINBLER, *Departments of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York*. It has been demonstrated, in the intact mammal, that glycine is the nitrogenous precursor of the protoporphyrin of hemoglobin. For further investigation of the mechanism of porphyrin formation, we sought a simpler biological system. We have found that whole blood of the duck can form heme from glycine *in vitro*. On aerobic incubation of whole duck blood with glycine labeled with 32 per cent  $N^{15}$ , isotopically labeled heme was synthesized. After four, seven, and twenty-four hours the  $N^{15}$  concentrations of the isolated heme were 0.051, 0.108 and 0.113 atom per cent excess  $N^{15}$  respectively. Further work is now in progress to determine the optimal conditions for the *in vitro* synthesis of heme, to determine the metabolites other than glycine which are necessary for this synthesis, and to study the chemical mechanisms and the enzymatic systems involved in this synthesis. In addition the use of this *in vitro* system is being extended to the investigation of protein and nucleoprotein synthesis. We had earlier found that the synthesis of heme occurs at an appreciable rate with the blood of patients with sickle cell anemia.

**Nature of the "sporogenes vitamin" and nutrition of *Clostridium sporogenes*** G. M. SHULL (by invitation), RICHARD W. THOMA (by invitation), and W. H. PETERSON, *Department of Biochemistry, University of Wisconsin, Madison*. The so-called "sporogenes vitamin" of Fildes, Pappenheimer, et al., is a growth factor required by *Clostridium sporogenes*. Moderate growth of eight strains of this organism has been obtained on a chemically defined medium containing amino acids, glucose, biotin, PAB, nicotinic acid, salts, buffer, and sodium thioglycolate. For heavy growth an unidentified factor, probably a polypeptide, is particularly effective. However, the unknown factor can be replaced by rather large amounts (about 25 mg per 10 ml) of arginine in combination with either tyrosine, phenylalanine or tryptophane (e.g. 20 mg per 10 ml). These amino acids appear to be preferential substrates, since they are metabolized with the production of ammonia even in the presence of glucose. Biotin is the only vitamin for which an absolute requirement exists. PAB and nicotinic acid exert a mild stimulatory effect on the growth of the organism. Oxybiotin can replace biotin but is required in about twice the concentration. Oleic, linoleic, ricinoleic, and vaccenic acids are active, in the order named,

in replacing biotin both in the basal medium and in an arginine-tyrosine medium. The use of emulsifying agents such as the Tweens greatly facilitates the demonstration of this phenomenon. Tween 80, an oleic acid ester, is active in high concentrations in replacing biotin.

It thus appears probable that either biotin or an unsaturated fatty acid, or both, were components of Pappenheimer's concentrates of the "sporogenes vitamin".

**The urinary excretion of amino acids and peptides after intravenous infusion to dogs** ROBERT H. SILBER, L. I. HOWE, and CURT C. PORTER (introduced by EDGAR G. MILLER), *Merck Institute for Therapeutic Research and the Merck Research Laboratories, Rahway, N. J.* Four protein hydrolysates and one amino acid mixture prepared in 5 different laboratories were given to dogs intravenously as the sole source of protein nitrogen daily for a period of 1 day, at a rate of 2 mg N per kg per min and a total dose of 120 mg N per kg. Only 5.5% of the amino acids in the amino acid mixture was lost in the urine whereas 15 to 29% of the hydrolysates was excreted in the form of amino acids and peptides. The amino acids of 3 protein hydrolysates were retained more efficiently than the peptides, with only 10 to 15% of the free amino acids appearing in the urine in contrast to 29 to 45% of the peptides. These hydrolysates contained 25 to 45% of their amino acids in peptide form.

The pattern of essential amino acids excreted in the urine after infusion of a peptide-free amino acid mixture was compared with the pattern of amino acids infused (6 mg N per kg per min and a total dose of 200 mg N per kg). The pattern of the infused mixture was then altered on the hypothesis that those amino acids which were excreted in disproportionately large amounts were present in excessive amounts in the mixture, and conversely, that those excreted in disproportionately small amounts were present in too low a concentration in the mixture. It has been possible in this way to obtain an amino acid mixture which more closely resembles the pattern of amino acids excreted in the urine. The significance of this will be discussed.

**The effect of hypertrophy on the chemical composition of rat cardiac muscle** LEONARD T. SKEGGS (by invitation), JACK R. LEONARDS (by invitation), and VICTOR C. MIERS, *Department of Clinical Biochemistry, Western Reserve University, Cleveland, Ohio*. The purpose of the present study was to determine the effect of cardiac hypertrophy on those chemical constituents which are known to play a major role in muscle contraction and also to evaluate chemically the presence of anoxia of the heart muscle. Cardiac hypertrophy was produced in rats by a variety of accepted

methods including hypertension, anemia and excessive administration of desoxycorticosterone acetate. These procedures increased the heart weight to body weight ratio by 40 to 75 per cent above normal. The hearts were then analyzed for inorganic phosphorus, phosphocreatine, the easily hydrolyzable phosphorus of adenosinetriphosphate and adenosinediphosphate, total acid soluble phosphorus, glycogen, and lactic acid. The presence of cardiac hypertrophy did not significantly change the concentration of any of the above mentioned constituents with the exception of the anemic animals in which there occurred a slight increase in lactic acid. If the cardiac muscle in hypertrophy was in a state of anoxia one would expect to find a marked increase in lactic acid and a fall in phosphocreatine and adenosinetriphosphate. Such changes were actually observed in the hearts of normal animals subjected to an atmosphere of 3 per cent oxygen for 5 minutes. It therefore appears that hypertrophy can exist in rat hearts without the accompaniment of detectable chemical changes. However, it is realized that marked changes may occur in the presence of a greater degree of hypertrophy or during heart failure and this phase is now being studied.

**The hydrolysis of glycylglycine and glycyl-L-leucine by peptidases.** EMIL L. SMITH, *Laboratory for the Study of Hereditary and Metabolic Disorders, and the Depts. of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City, Utah.* Although many dipeptides are hydrolyzed by animal tissues, the specificity and nature of the responsible enzymes have remained for the most part unknown. The hydrolysis of glycylglycine (GG) by extracts of rat muscle and human uterus is due to a specific dipeptidase which is strongly activated by  $\text{Co}^{++}$ , and to a lesser extent by  $\text{Mn}^{++}$ , but not by other divalent ions. This enzyme does not hydrolyze the tripeptide, glycylglycylglycine, or substituted compounds like benzoylglycylglycine, carbobenzoxyglycylglycinamide, benzoylglycinamide, glycylglycinamide, or glycylsarcosine. Sarcosylglycine is hydrolyzed although more slowly than GG. The hydrolysis of GG does not appear to parallel the hydrolysis of other dipeptides. The hydrolysis of GG follows the kinetics of a zero-order reaction. The maximal activity is at pH 7.6. The apparent dissociation constant of the cobalt-enzyme compound is  $2.8 \times 10^{-5}$  molar.

The glycyl-L-leucine (GL) hydrolyzing enzyme of rat muscle is activated by  $\text{Mn}^{++}$  that of human uterus by  $\text{Zn}^{++}$ . Maximal activity in both cases is obtained with phosphate buffer apparently because of the removal of  $\text{Ca}^{++}$  which acts as an inhibitor. With activating metal and phosphate, the hydrolysis of GL is a first order reaction, and is proportional to the enzyme concentration over a wide

range. The enzyme possesses the specificity of a dipeptidase since carbobenzoxyglycyl-L-leucine, carbobenzoxyglycyl-L-leucinamide, and glycyl-L-leucinamide are hydrolyzed either not at all or only very slowly by extracts rich in GL activity.

**The riboflavin requirement of the normal infant.** SELMA E. SNYDERMAN (by invitation), KATHERINE C. KETRON (by invitation), ANTHONY A. ALBANESE and L. EMMETT HOLT, JR. *From the Department of Pediatrics, New York University and the Children's Medical Service of Bellevue Hospital, New York City.* In determining the minimal requirement of the infant for riboflavin, it was not thought justifiable to induce symptoms of this deficiency. Instead, the attempt was made to ascertain accurately the riboflavin intake which would lead to minimal urinary excretion values. It has been clearly demonstrated in the case of thiamine, that when the intake is gradually reduced, the urinary excretion falls to a minimal level which is first reached at an intake two to three times as great as that which produces symptoms. There is evidence that a similar relationship occurs in the case of riboflavin. Infants were placed on metabolism frames and given purified diets in which practically all the riboflavin was provided as a weighed supplement. By gradual adjustment of the riboflavin ingestion over a period of months, the riboflavin intake that would just remain the minimal urinary excretion without additional spilling was determined. Maintenance at this level for many weeks produced no manifestations of riboflavin deficiency. This level, 0.40 to 0.45 mg per day would therefore seem to be an adequate one and presumably not far above the critical value. This intake was also checked by measurements of the riboflavin of the blood serum, the red blood cells, and the white blood cells. Only when the intake was dropped below the point of minimal urinary excretion were low blood values observed.

**Vitamin A absorption in newborns, older children, adults, and a storage in rats.** ALBERT E. SOBEL, LOTTIE BESMAN (by invitation) and BENJAMIN KRAMER. *Departments of Biochemistry and Pediatrics, The Jewish Hospital of Brooklyn, 16, N. Y.* Newborn children show impaired intestinal absorption compared to children above one year of age or adults, as indicated by low vitamin absorption curves after feeding Oleum Percomorphum (Vitamin A ester) or the nonsaponifiable fraction of fish liver oil (Vitamin A alcohol) dissolved in maize oil. On feeding Vifort, which is an aqueous dispersion of Vitamin A alcohol (containing the vitamin B complex, Vitamins C and D) elevated vitamin A absorption curves were observed. Upon administering an aqueous dispersion of vitamin A similar to Vifort, containing the same amount of dispersing agent (16% sorbitan monolaurate polyoxyalkalene derivative) but no other

vitamins a rise similar to that found with Vifort was obtained. Thus it appears that in newborn children there is an impaired absorption of Vitamin A in oil, which may be remedied when the vitamin A is given in an aqueous dispersion. Moreover, the % of dispersing agent and not the absence of vitamin supplements is the important factor in determining the magnitude of improved absorption. In older children and in adults there was also improved absorption with aqueous dispersions as measured by vitamin A tolerance curves.

Liver storage in rats was about 3 times as high with vitamin A in aqueous media (with or without supplements) than the same amount of vitamin A dissolved in oil.

**Influence of feeding amino acids on the spinal fluid level of free amino acids.** JAMES D. SOLOMON and STANLEY W. HILK (introduced by ORVILLE BERGIN) *Department of Biological Chemistry, University of Illinois College of Medicine and the Research Laboratories, The Wilson Laboratories, Chicago, Ill.* In a preceding abstract the levels of eleven individual free amino acids in cerebrospinal fluid were reported. The results of some experimentally produced changes in the concentrations of arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, valine, methionine, and cystine in the spinal fluid of human subjects are reported. The amino acids were fed orally in aqueous suspension 4 hours before collecting the spinal fluid by lumbar puncture. All were fed in combination with dl-isoleucine and in addition some were fed singly. It was found that there was a considerable variation in the rise of single amino acid from the average normal level. It was also observed that the effect of dl-isoleucine on the rise of other amino acids when fed in combination showed marked difference. For example, 25 grams of l(+)-arginine given with 15 grams of dl-isoleucine caused arginine to rise 80% and isoleucine to rise 311% above the average normal values. On the other hand, 25 grams of l(+)-histidine given with 15 grams of dl-isoleucine caused a rise in the histidine value to 304 per cent and the isoleucine value to 89% above the average normal values. When 25 grams of dl-methionine were given alone (12 hours before collection of sample) there was a rise of 325 per cent, while the ingestion of 25 grams of dl-valine alone (4 hours before collection of sample) the rise in the valine level was 900 per cent above the average normal level.

**A paradoxical effect of insulin on glucose assimilation.** MICHAEL SOMOGYI *From the Laboratory of the Jewish Hospital of St. Louis, St. Louis, Mo.* Insulin action is generally associated with an acceleration of glucose assimilation by the tissues (transfer of blood sugar into tissue cells). If a healthy person receives 50 gm. of glucose by mouth, a simultaneously administered small dose of insulin

(2.5-5 units) increases the rate of assimilation, both in the liver and in the peripheral tissues. This is reflected by the fact that the glucose-time (glucose tolerance) curve will run on much lower levels than if glucose alone is administered (Hims-worth). Our experiments are in line with this known fact. If, however, the same small dose of insulin is given during the postabsorptive state and is allowed to produce hypoglycemia, the assimilation of subsequently administered glucose is greatly inhibited instead of being accelerated. This fact was demonstrated in our experiments as follows. Healthy adult persons were given intravenously 1-5 units of crystalline insulin, and 30-60 minutes later were fed 50-100 gm. of glucose by mouth. Although at the moment of the ingestion of the glucose the blood sugar was at hypoglycemic levels, it invariably rose to much higher hyperglycemic levels than if the same amount of glucose was fed without the administration of insulin. Indeed, in some instances the hyperglycemia reached diabetic levels. This phenomenon is interpreted as follows. The hypoglycemia, in the present instance caused by insulin, mobilizes (activates) an insulin antagonistic mechanism which inhibits insulin action. This mechanism (the identity of which we shall not discuss at this occasion) does not stop at simply nullifying the action of the injected insulin, but—by over compensation—exerts a “diabetogenic” effect. Our clinical studies proved that recognition of this phenomenon has a far-reaching significance in the insulin treatment of diabetes mellitus.

**An enzymatic micromethod for determination of acetic acid.** MORRIS SOODAK (by invitation) and FRITZ LIPMANN *Biochemical Research Lab., Massachusetts General Hospital, and the Departments of Biological Chemistry and Medicine, Harvard School, Boston.*—In enzymatic acetylation of sulfanilamide in pigeon liver extracts, acetate may be made the limiting factor, cf. Kaplan and Lipmann, *Federation proceedings* 6, 266, 1947. This system may be adapted to quantitative determination of small amounts of acetate. For such purpose, it is necessary to use a purified enzyme. From acetone powder extract, an active preparation was obtained between 40 and 70 per cent saturation with ammonium sulfate. Care must be taken to eliminate traces of acetate usually present in ATP. Barium nitrate has to be used as precipitant for ATP-preparations used in this method. In such a system, between 3 and 12  $\mu$ g of acetate per 0.3 ml, the acetylation of sulfanilamide is proportional with the acetate concentration. In over hundred-fold concentration, propionate, butyrate, and valerate interfere with acetate determination.

A very interesting interference was observed with acetoacetate. It could be shown that the activity of acetoacetate is due to its preliminary



attack by an independent enzyme. Some application of the method in the study of acetate metabolism will be reported.

**The determination of choline in brain lipids**  
WARREN M. SPERRY and FLORENCE C. BRIND (by invitation) *Dept. of Biochemistry, New York State Psychiatric Inst., New York*. For the application of our micro method (Fed. Proc., 4, 104, 1945) to the determination of choline in brain lipids it was necessary to find an agent which would hydrolyze sphingomyelin and which could be removed, or treated so it would not interfere in the procedure. HI gave promise of satisfying these requirements, but the occasional low recoveries of choline, noted previously (Fed. Proc. 5, 155, 1946), were found to represent destruction of choline by HI. Glacial acetic acid appeared to cleave choline quantitatively from brain lipids, but the reaction is slow, requiring long heating in sealed tubes. Dilute  $\text{HNO}_3$ , suggested by Ducet and Kahane (Bull. soc. chim. biol. 28, 794, 1946), did not yield satisfactory results. With a saturated  $\text{Ba(OH)}_2$  solution there were large losses which were traced mainly to hydrolysis in glass apparatus and were presumably caused by traces of silica. Hydrolysis in monel tubes greatly improved the results, but recoveries were still about 10 to 15% low. The loss did not seem to represent a destruction of choline as it was more or less independent of the time of heating. When choline was heated in a half-saturated  $\text{Ba(OH)}_2$  solution, found by Dr. Jordi Folch-Pi to cleave choline from lipids (personal communication), there was still a small apparent loss (about 5%), but this can be corrected by suitable controls. Half-saturated  $\text{Ba(OH)}_2$  appears to satisfy reasonably well the requirements stated above, and to make possible the determination of choline in brain lipids with our method.

**Polarographic studies in cerebrospinal fluids**  
MORVA SPIEGEL-ADOLF and ARNOLD S. J. LEE (by invitation) *Dept. of Colloid Chemistry, Temple University School of Medicine, Philadelphia, Penna.* In a first series of experiments polarography was used for the study of some organic substances in the cerebrospinal fluid (CSF). Former studies (J. Nerv. Ment. Disease 89, 311, 1939) had ascertained quantitative and qualitative changes in pathologic CSFs escaping routine methods. A self-recording polarograph (Leeds and Northrup) was available. In preliminary experiments the findings of Gillam about the polarographic behavior of ascorbic acid could be confirmed. As little as 0.3 mg./100 ml. can be detected by this procedure, both in artificial mixtures sufficiently similar to CSF and in genuine CSFs. The presence of nucleic acids and derivatives does not appreciably influence these results. For the simultaneous determinations of ascorbic acid and nucleic acid

and derivatives a combination of polarographic, ultraviolet (D. U. Beckman photoelectric Quartz spectrophotometer) and chemical methods was used. It could be shown that the selective absorption at about 265 m $\mu$  is due to both nucleic acid and ascorbic acid as has been assumed previously by one of us (J. Phys. Chem. 50, 447, 1946). After the oxidation of ascorbic acid a part of the selective absorption subsides. Through colorimetric studies the presence of nucleic acid derivatives in the CSF of pathologic origin could be confirmed. In a final series of studies the colloidal power of CSF in suppressing the initial peak of lead chloride has been tested. Experiments are in progress to correlate these findings to the protein content and gold sol reaction of the CSFs.

**The metabolism of the  $\alpha$ ,  $\gamma$  and  $\beta$  hydrogen atoms of L-leucine**  
DAVID B. SPRINSON (by invitation) and D. RITTENBERG *Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*. The occurrence, in mammalian tissues, of an enzyme system capable of hydrolyzing dehydropeptides has been considered to support the view that dehydrogenation of proteins or peptides is a significant metabolic reaction. In order to investigate this possibility  $\alpha$ ,  $\beta$ ,  $\gamma$  deuterio-L-leucine containing  $\text{N}^{15}$  in the amino group was synthesized and fed to rats. L-leucine was then isolated from the body tissues. By appropriate degradative methods the concentration of deuterium at each of the labeled positions was determined in the compound fed and the one isolated. It was then possible to calculate the changes in D/ $\text{N}^{15}$  ratio for the  $\alpha$ ,  $\beta$ , and  $\gamma$  positions of the amino acid following interaction with the tissue proteins. The results indicate that the  $\beta$  and  $\gamma$  deuterium atoms are diluted about one-half as rapidly as the  $\text{N}^{15}$ . The  $\alpha$  deuterium atom, on the other hand, was removed three times as fast as the nitrogen. It, therefore, appears that the leucine could not have undergone extensive  $\alpha$ ,  $\beta$  dehydrogenation. The extraordinary lability of the  $\alpha$  hydrogen may be due to the influence of transaminase enzyme systems such as recently discussed by Konikova, Dobbert and Braunstein (Nature, 159, 67 (1947)).

**The concentration of the labile factor of prothrombin in the blood of various species**  
MARIO STEFANINI (by invitation) and ARMAND J. QUICK *Department of Biochemistry, Marquette University School of Medicine, Milwaukee*. When oxalated human plasma is stored, prothrombin activity decreases due to the disappearance of a plasma principle, which was originally named component A, but which has been renamed the labile factor. Since this principle is not adsorbed by  $\text{Ca}_3(\text{PO}_4)_2$ , plasma treated with this reagent retains its labile factor, but loses its prothrombin activity. When this plasma is added to stored plasma, it reduces

the prothrombin time to normal or even shorter. On this finding a quantitative test was developed for estimating the concentration of the labile factor in plasmas of different species. Oxalated human plasma was stored until its prothrombin time exceeded 35 seconds. The degree of delay does not significantly influence the test. The plasma to be tested was treated with  $\text{Ca}_3(\text{PO}_4)_2$ . One volume of this plasma was added to 9 volumes of stored human plasma and the prothrombin time determined. Since human plasma was found to contain the smallest concentration of the labile factor, it was made the standard for comparison. The plasmas from other species were diluted until they approximated the concentration of the labile factor in human plasma. The degree of dilution served as a measure of the relative concentration. On this basis, rabbit plasma contains 50, dog 10, cow and guinea pig 5 times as high a concentration as human plasma. It appears that the rapid loss of prothrombin activity of human plasma is attributable to its low content of the labile factor.

**Mechanism of hyperbilirubinemia due to sodium nicotinate.** MARIO SILIVINI (introduced by A. J. QUICK) *Department of Internal Medicine, University of Roma, Italy and Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin.* It has been found (Am J Med Sc, 213, 150, 1917) that the transitory rise of indirect reacting bilirubin of serum following intravenous injection of 30 mg. of sodium nicotinate intravenously in man is not due to exaggerated hemocatheteris or to mobilization of tissular deposits of biliary pigments. The problem was further studied by injecting sodium nicotinate in normal subjects while collecting as much bile as possible for two hours with continuous suction through a Rehfuess tube passed into the duodenum. The injection was followed by a remarkably copious flow of bile for about one hour. The serum bilirubin increased much less than when bile was not removed, this agrees with the finding that sodium nicotinate causes a minimal rise of serum indirect reacting bilirubin in patients with complete biliary obstruction. In biliary obstruction or when bile was removed from the duodenum, the transitory increase in urobilin excretion normally occurring within two hours after injecting sodium nicotinate was not observed. These experiments suggest that 1) sodium nicotinate injection is followed by hyperbilirubinemia only if bile is normally present in the intestine. The substance apparently stimulates the bile flow thus increasing the quantity of mesobilirubinogen available for reabsorption in the blood stream through the venous extraportal system (Blankenhorn, J Exp Med, 45, 195, 1927) as indirect reacting bilirubin. 2) mesobilirubinogen, serum indirect reacting bilirubin and urobilin are strictly related. 3) increased urobilinemia may

not always be due to pathological conditions but may result from changes in absorption of mesobilirubinogen.

**Chromatography of amino acids. Solvent systems for the fractionation of protein hydrolysates.** WILLIAM H. SELIN and STANFORD MOORE (by invitation) *Rockefeller Institute for Medical Research, New York City.* Employing potato starch columns (cf. Elsdon and Syngé, Biochem J, 38, Proc IX, 1911, Syngé, Biochem J, 38, 285, 1941), with the solvent system 1:1 butanol-benzyl alcohol-water, quantitative chromatographic methods have been developed for phenylalanine, leucine, isoleucine, tyrosine, and valine in protein hydrolysates (Moore and Stein, Annals N Y Acad Sciences, in press). Methionine has subsequently been rendered quantitative in this solvent. By the use of other solvents the technique has been extended to include additional members of the amino acid series. With a solvent system which gives more rapid band rates, a quantitative picture can be obtained which includes all of the amino acid components of a protein hydrolysate, with a few overlaps. For example, a single chromatogram (0.9 x 30 cm) run with 1:2:1 n-butanol:n-propanol:0.1 N HCl followed (after the emergence of aspartic acid) by 2:1 n-propanol:0.5 N HCl gives successive effluent peaks corresponding to the following components: leucine-isoleucine, phenylalanine, methionine-valine, tyrosine, proline, glutamic acid-alanine, threonine, aspartic acid, serine, glycine, ammonia, arginine, lysine, histidine, and cystine. This type of experiment has been used for screening work and has given quantitative values for those components which emerge as single peaks. The procedure has been simplified by the use of narrow columns about 1 cm in diameter. One-half cc. fractions are collected directly in colorimeter tubes on an automatic fraction collector and the entire fraction submitted to colorimetric ninhydrin analysis, thereby eliminating the pipetting of aliquots. About 2.5 mg. of protein are required per chromatogram.

**Synthesis of S-benzyl-thiopyruvic acid and its conversion to N-acetyl-S-benzyl-L-cysteine in the rat.** JAKOB A. STEKOL *The Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia, Pa.* S-Benzyl-thiopyruvic acid was synthesized from chloropyruvic acid and benzyl mercaptan, and characterized. One per cent of S-benzyl-thiopyruvic acid mixed with a complete casein diet was fed to four adult rats, and from the urine of these rats N-acetyl-S-benzyl-L-cysteine was isolated and identified by analysis and by comparison with the synthetic material and with a similar product which was excreted by the rats on administration of benzyl chloride or S-benzyl-L-cysteine. The results are proof for the conversion of S-benzyl-thiopyruvic acid to the

corresponding N acetyl-L amino acid derivative *in vivo*. The data presented, together with the earlier work, offer experimental evidence for the hypothesis expressed in regard to the possible formation of S benzyl-thiopyruvic acid as the intermediate in the inversion of S benzyl-D cysteine to N acetyl S benzyl-L cysteine *in vivo* (J Biol Chem, 124, 129 (1938), 128, 199 (1939)).

**Influence of the thyroid upon incorporation of deuterium into tissue constituents of the rat** DEWITT STETTIN, JR., and ABELL KARP (by invitation) Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York. Groups of young rats were maintained on a synthetic, fat free diet and were placed on a deuterium oxide regimen for 0.5, 1, 2, 4 and 8 days prior to being killed. The concentrations of deuterium were then determined in the glycogen of liver and of carcass, in the liver and depot fatty acids, in the cholesterol of liver and of carcass, in the liver protein, and in the body water. Three series of such experiments have been completed, first, a control series receiving no medication, second, a series in which the rats received thio uracil in their drinking water at a concentration of 0.1%, and, third, a series in which 1% of desiccated thyroid gland was added to the diet. From the rates at which the various tissue constituents became enriched with isotope, alterations in the rates of various anabolic processes produced by the medications employed could be inferred.

**Effect of zinc-hydrochloric acid hydrolysis on the estrogens in human urine** BENJAMIN F. STIMMEL, Rees-Stealy Medical Research Fund, San Diego, Calif. Previous work in this laboratory has shown that there is some chemical confirmation for Smith and Smith's observation that the enhanced estrogenic activity of urine extracts prepared by zinc hydrochloric acid hydrolysis is greater than can be accounted for by assuming conversion of estrone to  $\alpha$  estradiol and more complete hydrolysis. Further evidence has been derived, with our liquid chromatogram technique, from studies with human pregnancy urine and with non pregnancy urine from estrogen treated subjects which would indicate that zinc-hydrochloric acid hydrolysis (1) converts the major portion of estrone to estradiol, (2) yields estradiol titers which are much higher than can be accounted for by conversion of estrone to estradiol, (3) yields estriol titers which appear higher than can be accounted for by more complete hydrolysis. Subsequent zinc hydrochloric acid hydrolysis of residual urine after hydrochloric acid hydrolysis and ether extraction yields sufficient estrogen to account for a major portion, but not all, of the enhanced titer of zinc hydrochloric acid hydrolyzed urine. Zinc hydrochloric acid hydrolysis of the phenolic residue from hydrochloric acid hy-

drolysis caused almost complete destruction of the estrogen present. These observations would appear to offer additional evidence in support of the theory that hydrolysis of the urinary estrogens in the presence of a reducing medium rehydrogenates certain estrogen metabolites. These hypothetical metabolites appear in the estradiol and estriol fractions of our liquid chromatogram.

**Inhibition of pteroylglutamic acid conjugase by glutamic acid peptides of p-aminobenzoic acid** E. L. R. STOKSTAD, J. PIERCE (by invitation), T. H. JUKES and A. L. FRANKLIN (by invitation) Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. The action of glutamic acid peptides of p-aminobenzoic acid on the activity of a conjugase preparation which converted conjugated forms of pteroylglutamic acid (PGA) to free PGA was studied. It was found that p-aminobenzoyl gamma-glutamyl-gamma-glutamylglutamic acid at a concentration of 0.01 M reduced the activity of chicken pancreas conjugase by 80%. The tetraethyl ester of the same peptide produced no inhibition. P-nitrobenzoyl-gamma glutamyl gamma glutamylglutamic acid was only about half as active an inhibitor of conjugase as the corresponding p-aminobenzoyl compound. The activity of simpler peptides was also investigated.

**Interrelationships between pteroylglutamic acid, adenine, thymine, and antagonists of pteroylglutamic acid** E. L. R. STOKSTAD, M. REGAN (by invitation), A. L. FRANKLIN (by invitation), and T. H. JUKES Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. It was previously reported (Franklin, A. L., Stokstad, E. L. R., Belt, M. and Jukes, T. H., J Biol Chem, 169, 427 (1947)) that a crude "pteroylglutamic acid antagonist" showed competitive inhibition against pteroylglutamic acid (PGA) when measured with *Streptococcus faecalis* R as a test organism. Pteroylaspartic acid was found to show competitive inhibition against PGA (Hutchings, B. L., Mowat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and Subbarow, Y., J Biol Chem, 170, 323 (1947)) and the growth promoting effect of thymine was found not to be reversed by pteroylaspartic acid. In the present investigation a purified culture medium was used from which PGA, adenine and thymine were omitted. The crude antagonist was found to inhibit growth of *S. faecalis* R in the presence of the following combinations: PGA, PGA plus adenine, PGA plus thymine. However, when both thymine and adenine were added to the culture medium, growth was stimulated and the crude antagonist had no inhibitory effect. The effect of another PGA antagonist, N<sup>1</sup>-methylpterotic acid (Cosulich, D. B., and Smith, J. M., Jr., J Am Chem Soc, in the press), was

also studied in relation to purines and pyrimidines with *S. faecalis* R. Experiments were also carried out with rats and chicks. Thymine produced little or no growth or hematological response in rats maintained on a PGV free diet containing succinylsulfathiazole, nor was thymine able to protect rats against the deficiency syndrome produced by the crude antagonist. Thymine at levels of from 0.5% to 2% in the diet was ineffective in protecting chicks against PGV deficiency on a purified diet with PGV omitted.

**The role of acetylphosphate in the phosphoclastic and dismutation reactions of pyruvate.** HAROLD STRECKER (by invitation), L. O. KRAMER and HARLAND G. WOOD, *Department of Biochemistry, Western Reserve University, Cleveland 6, Ohio*. The occurrence of acetylphosphate as an intermediate in the phosphoclastic reaction ( $\text{CH}_3\text{COOOH} + \text{H}_2\text{PO}_4 \rightleftharpoons \text{CH}_3\text{COOPO}_3\text{H}_2 + \text{HCOOH}$ ) with *E. coli* is indicated by the formation of a labile phosphate compound and furthermore the reversibility of the reaction appears likely as judged by fixation of formate (Lipmann et al. and Utter et al.). Nevertheless, failure of numerous investigators to demonstrate activity of synthetic acetylphosphate with other enzyme preparations prompted further tests with *E. coli*. The following carbon labeled compounds,  $\text{C}^{13}\text{H}_3\text{COOH}$ ,  $\text{CH}_3\text{C}^{13}\text{OPO}_3\text{H}_2$ ,  $\text{HC}^{14}\text{OOH}$  were synthesized and added simultaneously to non labeled pyruvate which was fermented to partial completion. With our enzyme preparation acetate, formate, lactate and  $\text{CO}_2$  were products of the reaction indicating that part of the pyruvate underwent a dismutation reaction ( $2\text{CH}_3\text{COOOH} \rightarrow \text{CH}_3\text{CHOHCOOH} + \text{CH}_3\text{COOH} + \text{CO}$ ). The residual pyruvate of the fermentation was degraded by yeast carboxylase with formation of  $\text{CO}$  from the carboxyl group and acetaldehyde from the  $\alpha$  and  $\beta$  carbons. The carboxyl carbon contained a high concentration of  $\text{C}^{14}$  but there was no  $\text{C}^{13}$  in the  $\alpha$  and  $\beta$  carbons. Since carbon from  $\text{N}_2\text{HC}^{14}\text{O}_3$  was not fixed in significant amounts under the conditions of the experiment, the  $\text{C}^{14}$  of the formate apparently did not enter as  $\text{CO}_2$ . The results indicate that formate is fixed in pyruvate without occurrence of acetate or acetylphosphate as essential intermediates. It is concluded that either synthetic acetylphosphate is not an active component of the phosphoclastic reaction or there is another mechanism for the fixation of formate. It is considered possible that the chemically synthesized acetylphosphate is not identical with biological acetylphosphate.

**A comparison of reactions of cysteine and beta-beta dimethyl cysteine.** M. X. SULLIVAN, *Chemical Medical Research Institute, Georgetown University, Washington, D. C.* Over a period of years data have been given to indicate that the Sullivan test for cysteine requires the presence of free  $-(\text{SH})$ ,

$-(\text{NH}_2)$ ,  $\text{COOH}$  and in the arrangement in which these groups occur in cysteine. It was shown by Cutler in 1941 that mono methyl cysteine gave the characteristic color when Lugg's 1933 modification of the Sullivan test for cysteine was used. Carter, however, found that one isomer gave the same amount of color as an equivalent amount of cysteine while the other isomer gave only 20% as much color. While in this laboratory S methyl cysteine was found negative in the Sullivan cysteine reaction it should be expected no study was made as to the effect of substitution on the alpha or the beta carbon. Recently, however, we had occasion to study beta beta dimethyl cysteine and found it absolutely negative in the cysteine reaction and also negative in the cystine procedure. However, in the cystine procedure it gives a positive reaction up to the addition of  $\text{Na}_2\text{S}_2\text{O}_4$ , that is it behaves like cystine. Data will be given as to the reason for the negative Sullivan reaction and for possibility of testing for the beta beta dimethyl cysteine as an entity and as a decomposition product of penicillin.

**Effect of pH on the aerobic metabolism of lymphosarcoma cells.** WILLIAM H. SUMMERSON, HELENA GARDER (by invitation) and JOHNNA M. LEF (by invitation), *Department of Biochemistry, Cornell University Medical College, New York, N. Y.* The aerobic metabolism of the Gardner mouse lymphosarcoma cell has been studied *in vitro* in bicarbonate containing media over the pH range 6.9 to 7.6. At pH 7.4, oxygen consumption averaged 0.80 microliters per  $10^6$  cells per hour, and R.Q. averaged 0.83, neither value showed any marked alteration with change in pH. By contrast, aerobic acid production proved to be dependent upon pH in a substantially linear fashion, with values ranging from 0.30 microliters of acid per  $10^6$  cells per hour at pH 6.9 to 1.05 microliters at pH 7.6. All of the acid produced aerobically by this tumor cell is lactic acid. Glucose utilization by the cell is substantially accounted for in terms of lactic acid produced. Hence aerobic glucose utilization is likewise a function of the pH of the medium, and the substrate for oxidative metabolism is not exogenous glucose. The bearing of these findings on current concepts of tumor metabolism will be discussed.

**Influence of the concentration of the vitamin B complex on protein utilization.** BARNETT SURE and FREELAND ROMANS (by invitation), *University of Arkansas, Fayetteville*. Seven concentrations of the vitamin B complex were used. These concentrates were fed to albino rats separately from the ration. The daily doses of thiamine, riboflavin, pyridoxine, and niacin varied from 3 to 100  $\mu\text{g}$ . The doses of calcium pantothenate ranged from 25 to 600  $\mu\text{g}$ , those of p-aminobenzoic acid, from 0.25 to 12 mg, inositol, from 0.075 to 3 mg, and choline chloride, from 3 to 12 mg. Three levels of purified casein

(Smilo) were used, as follows 71% and 87%, supplemented with 25 mg cystine daily, and 173%, also, 9% lactalbumin (Borden). On the 71% and 87% levels of casein intake and on the 9% lactalbumin ration the optimum protein utilization was obtained on the concentration of the vitamin B complex, which furnished the following daily doses, 3  $\mu$ g thiamine, 3  $\mu$ g pyridoxine, 3  $\mu$ g niacin, 25  $\mu$ g calcium pantothenate, 250  $\mu$ g p aminobenzoic acid, 75  $\mu$ g inositol, and 3 mg choline chloride. On the 173% protein level, no notable differences were found until after the animals were ten weeks on such diet, when again the same low concentration of the B complex proved to be the optimum. The greatest transition occurred on the 71% casein level when changed from 3 to 5  $\mu$ g daily doses of thiamine, riboflavin, pyridoxine, and niacin, and when the rest of the components of the vitamin B complex was doubled. The biological value of the casein was thus increased from 48.5 to 85.5.

**A glycogenolytic factor from pancreas.** EARL W. SUTHERLAND and CHRISTIAN DE DUVE (introduced by CARL F. CORI) *Dept of Biochemistry, Washington Univ School of Med, St Louis*. Liver glycogenolysis has been reported previously to be stimulated *in vivo* and *in vitro* by a non dialyzable factor which is present in some insulin preparations and which, unlike insulin, withstands incubation with dilute alkali. Extracts of pancreatic tissue from dogs, rabbits and cattle, prepared by a method which is known to extract insulin, were found to increase glycogenolysis in liver slices. Maximal activity in the test system was obtained with amounts of extracts representing 0.05 to 0.20 grams of pancreas, addition of more glycogenolytic factor did not further increase glycogenolysis. The activity was retained after dialysis and after alkali incubation. Extracts of spleen, liver and kidney were completely inactive. Slight activity was found in duodenal extracts. Extracts of fetal calf pancreas showed maximal activity in amounts equivalent to 0.02 to 0.04 grams. Pancreatic extracts from two alloxan diabetic rabbits showed essentially the same activity as those from normal rabbits and caused hyperglycemia without subsequent hypoglycemia when injected intravenously into a rabbit. Some material showing similar activity was found to be present in concentrated perfusates of isolated dog's pancreas and, in some cases, in alcoholic extracts of lyophilized dog's serum. Good recovery of the glycogenolytic factor was obtained when a small amount of an insulin preparation containing the factor was treated by these methods and no activity arose from similar treatment of an insulin preparation free of the factor.

**An enzyme requiring DPN involved in the metabolism of testosterone by liver tissue.** MAX L. SWEAT (by invitation) and L. T. SAMUELS *Department of Biochemistry, University of Utah*

*School of Medicine, Salt Lake City, Utah*. An active cell free extract which will metabolize testosterone has been prepared from rat liver. The rate of the reaction is enhanced by the addition of diphosphopyridine nucleotide. It has been found that both malate and lactate inhibit the destruction of testosterone. The addition of nicotinamide increases the rate of destruction. The inhibition produced by these two DPN-requiring substrates, together with the increased rate of destruction in the presence of nicotinamide is strong evidence that the testosterone destroying enzyme is another enzyme which required DPN as a co factor. The products of the reaction in which DPN is a co enzyme appear to be 17 ketosteroids without a conjugated double bond system. Work on the further purification of the enzyme is in progress.

**A procedure for the determination of 1-aminosalicylic acid in blood and urine.** DAVID M. TAYNENT and MARGARET L. LELAND (introduced by RANDOLPH T. MAJOR) *Merck Institute for Therapeutic Research, Rahway, N. J.* A procedure has been developed for the determination of microgram amounts of 4-aminosalicylic acid in blood and urine. This depends upon two suitable color reactions which have been studied. The color produced is red when the drug is coupled in acidic solution with diazotized p-mitraniline followed by the addition of sodium hydroxide, and blue when the drug and p-mitraniline are both diazotized, followed by coupling in the presence of pyridine and then by addition of sodium hydroxide. The colors due to interfering substances in urine and in blood filtrates are equal in intensity with either procedure and their effect can be eliminated by taking two samples for the determination, developing the red color in one and the blue in the other, and reading in the colorimeter at 620 m $\mu$ , using the former as the blank for the latter. In practice the same reagents are used in both reactions, and the color developed depends upon the order in which they are added. Amounts of 4-aminosalicylic acid between 1 and 15 micrograms in 4 ml of solution can be determined within  $\pm 5\%$  by this procedure. It is applicable to blood filtrates prepared with tungstic acid and to urine dilutions.

**Some factors which influence methionine excretion in the rat.** HERBERT C. TIDWELL (introduced by MAX N. HUFFMAN) *Department of Biochemistry, Southwestern Medical College, Dallas, Texas*. The effect of changes in the protein and fat content of the diet, of fasting, and of the injection of methionine and choline on the urinary excretion of methionine has been investigated. Variations in the dietary protein during 4 or 33 days, with methionine intakes of 6 to 60 mg per 100 gm body weight, did not alter the methionine excreted. Unlike the results obtained by Homburger (*Am J Med Sci* 212, 68, 1946) on normal men, similar

excretion values were obtained on all these animals after injecting supplements of the free amino acid equal to one or two times the normal daily intake. Less methionine was excreted after 15 or 10% fat diets than a 5% one. Still less was excreted after a 3 day fast. However, again a like amount of the injected supplement was excreted whether the animals were fasted, or on the high fat diets. The injection of choline during the 3 day fast was accompanied by an increased methionine excretion. The decreased excretion after higher fat intakes, or fasting, might result from an increased need of lipotropic substances associated with an increased fat metabolism. The excretion of a similar amount of the injected amino acid, whether a deficiency existed or not, suggests that increased needs are not reflected by a marked decrease in excretion of the supplement. The small increases in excretion following these supplements indicate that the amino acid is largely utilized, either as the amino acid or some degradation products.

**On the bacterial metabolism of lysine** GERRIT TOENIES and DOROTHY L. GALLANT (by invitation) *Lankenau Hospital Research Inst and Inst for Cancer Research, Philadelphia 30, Pa.* If in our experimental medium for the determination of amino acids with *S. fecalis* (*Fed Proc*, 6, 298 (1947)) the concentration of lysine is suboptimal initial growth, which is proportional to the available lysine, is followed by rapid lysis of cells. An excess of 5% of lysine per cc is sufficient to prevent lysis. In the lysine limited medium lysis is prevented by omission of 98% of the phosphate buffer (0.3 M, pH 6.5), or its replacement by citrate buffer or sodium chloride of equal ionic strength. The irreversibility of the deamination of lysine (Schoenheimer) may explain the necessity of an excess of this particular amino acid for bacterial maintenance if phosphate accelerates amino acid exchange metabolism. However, lysis does not result from an interaction between cells and the lysine-deficient high-phosphate medium but expresses a property of cells grown in such a medium.

Gale (*J Gen Microbiol*, 1, 53 (1947)) has demonstrated in another strain of *S. fecalis* that normal cells contain substantial amounts of free lysine while bacteria grown in a medium of low lysine content contain very little of the free amino acid. A similar deficiency in internal free lysine would explain the difference between our cells grown in a high-phosphate medium in the presence or absence of excess lysine. A medium containing only salts, lysine and glutamic acid prevented lysis, in accordance with Gale's finding that such a medium enables lysine-deficient cells to imbibe lysine. We also confirmed that glucose cannot replace glutamic acid.

**The influence of KCN and pteroylglutamic acid on growth and porphyrin production of *Corynebacterium***

*terium hoffmannii* JOHN R. TOTTER and EDITH S. SIMS (by invitation) *Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock.* The organism *Corynebacterium hoffmannii* was grown in a basal medium complete for *Streptococcus faecalis* except for omission of pteroylglutamic acid (PGA). As supplement sterile KCN neutralized with acetic acid was added aseptically up to concentrations of 0.001, 0.002, and 0.003%. PGA was added to the medium before sterilizing in concentrations of 0.0, 0.005, 0.05, 0.1, and 0.2 mg per cc. Porphyrins were extracted and determined by the customary procedures after 5-8 days incubation at 32°. Growth was determined by measuring optical density at suitable intervals. The KCN retarded the growth somewhat but did not completely inhibit growth even at 0.005% concentration. The porphyrin production was diminished by 7% in the 0.001% KCN, by 20% in the 0.002% KCN, and by 70% in the 0.003% KCN when measured on equal volumes of culture suspensions with the same optical density and compared with a control. PGA at concentrations above 0.05 mg per cc counteracted the effect of KCN on both growth and porphyrin production. Accelerated growth was also seen at relatively high PGA concentrations in the medium without KCN. The actual concentration at which PGA became effective varied considerably for reasons not yet ascertained.

**The influence of fat in the diet upon nitrogen metabolism and liver protein regeneration** HARRY M. VARS and CHARLES E. FRIEDGOOD (by invitation) *Harrison Dept of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.* In a previous study (*Fed Proc*, 6, 257, 299), the diets used were made to contain only 3% of fat, 7% of the calories. From the work of Forbes, Deuel, and of others, the inclusion of adequate amounts of fat in the diet appears to improve the utilization of energy and the physical fitness of normal animals. Therefore, we have investigated the effect of adding 10% and 30% fat to a non-protein diet and to diets containing casein, upon liver protein regeneration and N metabolism after partial hepatectomy. Groups of rats were depleted of protein stores by a 2 week period of non-protein feeding, and 69% of their livers removed. They were then fed diets containing 10% and 30% fat for 2 weeks. Total N balances and new liver protein formed were determined. The diets so obtained have been compared with the earlier observations. An increased percentage of fat calories in the non-protein diet did not increase conservation of N as measured by liver protein regeneration, although it decreased the negative N balance. Fat added to a casein diet in which the protein to fat caloric ratio remained constant, caused a greater food intake with an associated increase in liver protein regeneration and N balance. This was also true in

unoperated controls. Ingestion of in mice used percentage of calories is fit during a period of isonitrogenous isocaloric feeding produced an increase in liver protein regeneration. This was accompanied by less retention of N by the body.

**An electrophoretic analysis of the interaction of aldolase and glyceraldehyde phosphate dehydrogenase with phosphate ions** SIDNEY F. VELLICK *From the Department of Biological Chemistry, Washington University School of Medicine*. Both aldolase and glyceraldehyde phosphate dehydrogenase contain an excess of basic over free acidic groups. If the individual groups were to exhibit dissociation constants in the range usually observed in other proteins one would predict alkaline isoelectric points whereas both proteins in phosphate buffers of ionic strength 0.1 are isoelectric on the acid side of neutrality. Since the substrates of both enzymes are anions one would attribute to them an affinity for anions if their kinetics were formulated in terms of an enzyme-substrate complex. The acid isoelectric points can be explained by the binding of phosphate ions. Electrophoretic analysis shows that the isoelectric point of aldolase shifts from 6.7 to 4.94 in phosphate buffers ranging from ionic strength 0.05 to 0.2. The dehydrogenase shows a similar variation. The bound charge as a function of pH and ionic strength can be calculated and dissociation constants can be derived. Other properties of the dehydrogenase are consistent with its tendency to bind anions.

**In vitro utilization of glucose by rat diaphragm muscle** C. A. VILLEE, F. M. STREX, and A. K. SOLOMON (introduced by A. B. HASTINGS) *Department of Biological Chemistry and the Biophysical Laboratory Harvard Medical School*. The experiments of Gemmell, Stadie, and Krahl and Cori have demonstrated an effect of insulin on the formation of glycogen by isolated diaphragm muscle. Experiments were undertaken to study the oxidation of glucose by diaphragm muscle *in vitro* under varying experimental conditions. Glucose labeled with  $C^{14}$  in positions 3 and 4 was made biosynthetically as glycogen in rabbit liver slices from pyruvate and  $C^{14}O$  by the Buchanan and Hastings method. Rat diaphragm muscle was incubated in Warburg vessels in a medium of 0.04 M sodium phosphate, 0.005 M  $MgCl_2$ , 0.08 M NaCl, and 0.2 per cent glucose, initial pH 6.8. A half diaphragm was placed in each vessel, to one of each pair of vessels, 0.5 units insulin per ml medium was added. The  $CO_2$  collected in the center well was precipitated as  $BaCO_3$ , plated and counted. Samples of the media before and after incubation were analyzed for glucose, and the glycogen in the diaphragm after incubation was hydrolyzed and determined as glucose.

In normal diaphragm muscle, from 50 to 80 per

cent of the glucose which disappears from the medium is oxidized, the remainder is converted to glycogen. The addition of insulin increases both glucose oxidation and glycogen formation, although there is no overall increase in respiration. The rates of glucose oxidation by muscle from alloxan-diabetic and from adrenalectomized rats are significantly lower and higher, respectively, than normal. These results support Cori's views that insulin affects primarily the hexokinase reaction.

**Studies on the composition of nucleic acids** ERNST VISCHER (by invitation) and ERWIN CHARGAFF *Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*. The method for the micro partition chromatography of purines presented recently (E. Vischer and E. Chargaff, *J. Biol. Chem.*, **168**, 781 (1947)) has been extended to the pyrimidines, cytosine, uracil, and thymine. It now permits not only the identification of the nitrogenous constituents of nucleic acids but also their quantitative estimation in minute amounts (5% of the individual component) with an accuracy of  $\pm 4\%$ . For the complete analysis of a nucleic acid 8 mg were employed, but even this small sample weight can doubtless be reduced. A study of the acid hydrolysis of yeast ribonucleic acid showed that while for the estimation of purines the customary treatment with dilute acid could be employed, the pressure hydrolysis with 20% HCl for the quantitative liberation of pyrimidines was not applicable since it resulted in an extensive conversion of cytosine to uracil. Formic acid could, however, be used. Two different solvent systems served for the separation: a mixture of butanol, morpholine, diethylene glycol, water for the purines, butanol-water for the pyrimidines. Results obtained by the use of this technique will be presented for a representative series of nucleic acids, viz., the pentose nucleic acids of yeast and pancreas and the desoxy-pentose nucleic acids of yeast, tubercle bacilli, thymus, and spleen.

**The inhibition of glutaminase by glutamic acid** HEINRICH WAELSCH and PHYLLIS OWADES (by invitation) *Depts of Biochemistry, New York State Psychiatric Institute and Columbia University*. Glutamine appears not to participate in certain metabolic conversions in which glutamic acid is implicated. In the tissues studied the amide occurs in considerably higher concentrations than the parent amino acid and its formation and breakdown may be one of the regulating mechanisms of glutamic acid metabolism. The glutaminase of brain or kidney is inhibited by glutamic acid (Krebs). This inhibition is dependent on pH, being smallest in the range of optimal enzyme activity. At pH 6.8 (0.1 molar phosphate) 0.015 molar glutamic acid inhibits the splitting of 0.004 molar glutamine by rat brain homogenate by about 90

per cent and at pH 7.9 by about 15 per cent. It has been shown that the glutaminase of brain is active only in the presence of certain anions such as phosphate, sulfate, arsenite (Carter and Greenstein) or citrate. The extent of the inhibition of glutaminase by glutamic acid is dependent on the concentration of phosphate (or sulfate or citrate), also in the pH range of optimal enzyme activity a strong inhibition can be obtained at low phosphate (0.025 molar) concentrations. Variations in the pH or in the phosphate concentration may therefore determine the rate of enzymatic liberation of glutamic acid from glutamine.

**Serine content of purothionin.** M. K. WALDEN (Introduced by A. K. Balls) *Enzyme Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA, Albany, California.* The protamine-like substance obtained from wheat flour is a crystalline hydrochloride by Bills, Hale and Harris (Cereal Chem., 19, 279 (1912)) has been found to contain serine. The presence of serine supports the idea that in nature purothionin is linked with a phospholipid which is responsible for the solubility of the mother substance in petroleum ether and the loss of that solubility, as well as of associated phosphorus, on hydrolysis to purothionin.

After hydrolysis of the recrystallized hydrochloride with constant boiling HCl for 20 hours, formaldehyde equivalent to 9.8 percent of serine (by weight) was obtained by the method of Boyd and Logan (Jour. Biol. Chem., 146, 279 (1912)). A phosphotungstic acid separation after Van Slyke, Hiller and MacFadyen (Jour. Biol. Chem., 141, 681 (1941)) excluded the possibility of hydroxylysine, 91 percent of the expected serine was found in the filtrate. No ethinolamine was found by the method of Blum (Biochem. Zeits., 305, 129 (1940)) and the residue remaining gave formaldehyde equivalent to 9.5 percent serine in the original substance. No formaldehyde was produced by periodate from the unhydrolyzed material. Arginine, serine and cystine thus account for more than half of the total nitrogen of the substance, and occur, respectively, in proportions of equivalents close to 2:2:1.

**Inhibitors of choline acetylase.** MICHAEL S. WEISS, HELEN VORPILIEFF, and DAVID NACHMANSOHN (Introduced by H. T. Clarke) *Dept. of Neurology, College of Physicians and Surgeons, Columbia University, New York.* The accelerating or inhibitory effects of some intermediate compounds on enzymes depends frequently on the degree of purity. Apparent contradictory observations of this type of effects are often due to the use of different enzyme preparations. Such effects may be the result of the reactions with other intermediate metabolic processes and not with the enzyme system itself.—Citric and L-glutamic acid increase the rate of freshly prepared and dialyzed

choline acetylase. No effect is observed with glutamic acid in active enzyme solutions prepared from acetone dried powder in presence of active coenzyme. The effect of citric acid seems to change with purification. In highly active solutions prepared from the head ganglion of Squid in which one gram of protein formed 150 milligrams of acetylcholine per hour, citric acid decreased the rate of formation in  $10^{-2}$ M concentration by about 50 per cent. The effect may be due to the removal of magnesium necessary for transphosphorylation  $\alpha$ -keto glutamic acid which like other  $\alpha$ -keto acids inhibits all preparations obtained so far from mammalian brain, had no effect on the Squid ganglion preparation.

Among the inhibitors of choline acetylase which are not naturally occurring compounds, the naphthoquinones are of particular interest. A considerable number of them, for which we are greatly indebted to Professor Louis I. Fieser, were tested and some of these effects will be described. The 2-Methyl-1,4-naphthoquinone-8-sulfonic acid (K salt) was found to have the same effect whether tested on the mammalian brain or Squid ganglion preparation, suggesting in this case an affinity to the enzyme system itself.

**Ozonolysis of the pyo compounds.** IBERT C. WELLS (by invitation), WILLIAM H. ELLIOTT (by invitation), SIDNEY A. FRAYER and EDWARD A. DOISEY *Laboratory of Biological Chemistry, Saint Louis University School of Medicine, Saint Louis, Missouri.* As a continuation of our work on the structure of the antibiotic substances produced by *Pseudomonas aeruginosa* (J. Biol. Chem., 159, 725 (1945)), some of them have been subjected to ozonolysis. P<sub>yo</sub> III ( $C_{15}H_{11}NO$ ) yielded one mole of nonenal (isolated as the 2,4-dinitrophenyl hydrazone, m.p. 106–107°C). No other products were isolated. P<sub>yo</sub> Ic (tetrahydro P<sub>yo</sub> III) treated in the same manner yielded no aldehyde or ketonic fragments but did produce N-caprylanthranilic acid, capric acid and capramide. These compounds were identified by ultimate analyses and comparison with appropriate derivatives. Anthranilic acid was detected spectroscopically but was not isolated. The yield of N-caprylanthranilic acid and the estimated yield of anthranilic acid accounted for one mole of N-caprylanthranilic acid per mole of P<sub>yo</sub> Ic. Octahydro P<sub>yo</sub> Ic (dodecahydro P<sub>yo</sub> III) produced upon ozonization one mole of capramide and a small amount of a new compound which is acidic, melts at 131–132°C, does not absorb ultra violet light and analysis indicates the formula  $C_{15}H_{35}NO_4$ .

**Acetaldehyde metabolism in dogs maintained on a purified diet.** W. W. WESTERFELD and J. M. MCKIBBIN (by invitation) *From the Department of Biochemistry, Syracuse University College of Medicine, Syracuse, New York.* Young adult dogs



were maintained on a purified diet containing casein 19, cod liver oil 2, cottonseed oil 7, salts 1, sucrose 28, glucose 10, and adequate amounts of thiamine, riboflavin, pyridoxine, pantothenic, choline, nicotinic acid and tocopherols. Within 4 months approximately 50% of the dogs developed a defect in acetilcholine metabolism that was brought out by a load test in which 80 mg. ACh per kg. were administered intravenously and its rate of disappearance from the blood was measured under standardized conditions. A delayed disappearance curve was not associated with weight or skin changes, but was associated with a liver pathology histologically similar to hydropic degeneration and identical with that attributed by Elman and Hefetz (J. Exp. Med., 73, 417, 1911) to protein deficiency. Other evidence of liver damage was not prominent, liver fat was normal, brom. sulfalein excretion and prothrombin times were normal, serum alkaline phosphatase may have been slightly elevated.

Once this defect was produced, it was difficult to restore the acetilcholine disappearance curve to normal by dietary means. The only pure vitamin that gave a positive response was inositol, and this effect was very erratic, it produced a beneficial response in only some of the dogs, and the response was often temporary. Negative tests were obtained with biotin, folic acid, p-aminobenzoic acid, vitamins C, K, and E, and with various combinations of these factors. Disappearance curves in some of the dogs could not be restored to normal even by feeding natural foodstuffs, such as meat, liver, milk, yeast, or molasses.

The effect of streptomycin on deamination and oxygen consumption by resting cells of *E. coli* KENT WIGHT (by invitation) and DEAN BIRN, National Cancer Institute, National Institute of Health, Bethesda, Maryland Warburg technique studies were carried out on the mode of action of streptomycin on resting cells of *E. coli*, strain NIH 119, grown 16 to 24 hours in nutrient broth at 37°C, centrifuged, washed and resuspended in 0.05 M phosphate at pH 7.4, and then studied 4 to 5 hours at 37°C, with and without addition of 10 to 20 p.p.m. streptomycin 0.012 M amino acid, or 0.007 M non nitrogenous organic acid.

Anaerobically, in  $N_2$ , the washed, resting *E. coli* cells deaminated aspartic acid and serine, but not threonine, alanine, or histidine. Streptomycin inhibited this ammonia production up to 50% in the case of aspartate and 35% with serine. No  $CO_2$  production was detected with any of the five amino acids, even in the presence of 0.01-0.02 M semicarbazide. Oxaloacetate and pyruvate, but not acetate, yielded  $CO_2$ , without inhibition by streptomycin, however.

In air, ammonia production from aspartate and serine was somewhat greater than in  $N_2$ , and the percentage inhibition by streptomycin was some-

what less (20-30%). At the same time, oxygen consumption ( $QO_2 = ca. 30$ ) was decreased about 50% by the fourth hour by streptomycin. Threonine and alanine gave the same oxygen consumption as aspartate, but less  $NH_3$  production, and no inhibition of either process by streptomycin. The  $QO_2$  with fumarate was decreased by streptomycin from 10 to 25, and with oxaloacetate from 90 to 50. However, streptomycin did not inhibit either the aerobic or anaerobic assimilation of ammonia when added with fumarate, the presumed end product of aspartate decarboxylation.

Variables affecting the assay of testosterone propionate using male castrate rats G. A. WILLS, SYBIL RAYMOND (by invitation) and L. I. PUGSLEY, Food and Drugs Division, Dept. National Health and Welfare, Ottawa, Canada. A study was made of the method of administration and of the time after castration on the precision and sensitivity of the method of assay of testosterone propionate described by Matheson and Hays (Endocrinology 37, 275, 1945). It was found that the standard error of the assay and the dose required to give comparative responses was considerably decreased by using two injections intramuscularly instead of the one injection subcutaneously recommended by Matheson and Hays. It was confirmed that, providing two weeks were allowed for the regression of the seminal vesicles, the response was independent of the time after castration.

The effect of biotin on mitotic activity in the mouse liver J. WALTER WILSON and ELIZABETH H. LEDUC (introduced by P. H. MITCHELL), Brown University. Biotin deficiency was produced in mice by inclusion of 20% dried egg white in a semi-synthetic diet. Five week mice placed on this diet showed signs of deficiency in about 5 weeks, 16 day mice in 3-4 weeks. During the development of the deficiency the mice grew rapidly and in the younger ones mitotic activity was observed in the liver. Mice with well developed symptoms showed practically no mitosis in the liver. Deficient mice kept on the diet were treated with a single injection of homologous liver and sacrificed daily. Mitotic activity in their livers was observed on the 4th and 5th days (mitotic rates 1.41 and .95). In others injected daily with 1  $\gamma$  of biotin intraperitoneally, or 2  $\gamma$  of biotin subcutaneously, mitotic activity was found in the 1st and 2nd days (mitotic rates .21 and .79). In still others transferred to the same diet with the egg white replaced by casein, mitotic activity was observed from the 2nd to the 6th day with the highest rate on the 4th (mitotic rate .65). Non deficient adult (15 week) mice were injected daily subcutaneously with 2  $\gamma$  of biotin and one sacrificed each day. Mitosis was observed on the 3rd day (mitotic rate .95). There is thus a falling off of mitotic activity in the liver with actual development of symptoms of biotin deficiency, and a significant increase after treat-

ment of deficient animals with liver or biotin or simply after injection of biotin into normal adults

**Incorporation of  $C^{14}$  labeled glycine into the protein of tissue homogenates** THODORI WINICK, FELIX FRIDBLERG (by invitation), and DAVID M. GREENBLERG *Division of Biochemistry, University of California Medical School, Berkeley* The incorporation of radioactive glycine into proteins was observed when homogenates of such tissues as rat spleen, liver, and kidney were incubated at 37° and pH 7.6 in the presence of the  $C^{14}$ -labeled amino acid. The process requires the presence of inorganic salts, oxygen, and certain organic metabolites, such as glucose and citrate. It is inhibited by azide or cyanide, and abolished by heating to 100°. In the case of spleen homogenates, the quantity of labeled glycine taken up per mg. protein was found to be proportional to both the glycine concentration in the medium and the reaction time. The activity of the homogenates was apparently associated with insoluble cell particles, which could be collected by centrifugation. Evidence that the process of glycine uptake represents an actual incorporation into the protein structure was provided by hydrolytic experiments. Following hydrolysis of the radioactive proteins with either proteolytic enzymes or hydrochloric acid, the  $C^{14}$  could be accounted for as glycine. The radioactivity of this glycine (recovered with the aid of carrier) corresponded fairly well to the  $C^{14}$  content of the initial proteins. It was estimated that approximately 0.2 per cent of the glycine residues of spleen proteins were replaced per hour by the labeled glycine added to the homogenate.

**Thyroxine activity and antagonism of some structural analogues of thyroxine** RICHARD J. WINZLER and EARL FRIEDEN (by invitation) *Department of Biochemistry and Nutrition, the University of Southern California School of Medicine, Los Angeles* A number of compounds structurally related to thyroxine have been prepared. These have included the dl-glycine homologue of thyroxine (3/5-diido-4-(3',5'-diido-4'-hydroxyphenyl) phenyl glycine) and the benzoic acid analogue of thyroxine (3/5-diido-4-(3',5'-diido-4'-hydroxyphenyl)benzoic acid) in which the side chain of thyroxine has been modified, and also dl-O-benzyl-n acetyl 3,5 diiodotyrosine in which the prime ring has been altered. These compounds have been tested both for thyroactivity and for thyroxine antagonism using their effect on tadpole metamorphosis, their effect on basal metabolic rates in rats, and their influence on the weight of of thyroid glands of thiouracil-fed rats. The results indicate that the glycine homologue of thyroxine possesses about 10% of the activity of dl-thyroxine when measured with tadpoles but has been inactive in the rat at levels up to 1.5 mg./kilo/day. No thyroxine antagonism has been evident in studies with this compound. The benzoic acid

analogue of thyroxine has been found to show about 20% of the activity of dl thyroxine in tadpoles, but has been inactive in rats up to levels of 5 mg./kilo/day. The observations of Woolley (1) on the antagonistic action of O-benzyl-n acetyl diiodotyrosine toward thyroxine in tadpole metamorphosis have been confirmed and extended. However, levels up to 50 mg./kilo/day showed neither thyroxine activity nor antagonism in rats.

**A spectrophotometric method for nicotine in blood** WILLIAM A. WOFF and MARINA HAWKINS (by invitation) *Tobacco Research Lab., Bowman Gray School of Medicine, Wake Forest College, Winston Salem, N. C.* A colorimetric procedure for nicotine has been adapted for use with human blood and modified to increase its sensitivity and to eliminate the "nicotine blank" inherent in certain other methods. Blood proteins are precipitated with trichloroacetic or metaphosphoric acids and the filtrate is divided into aliquots, each equivalent to 10 ml. blood. One aliquot of filtrate is saturated with NaCl, made 20% with NaOH, and steam distilled in an all glass apparatus. The color value (nicotine plus "blank material") of the distillate is determined by the cyanogen bromide-B-naphthylamine reaction. The resulting color is compared with that of a nicotine standard similarly treated, both read at 490 millimicrons in a Beckman spectrophotometer. A second aliquot of acid filtrate is treated with an activated carbon which removes the nicotine and leaves the "blank material" in solution. The treated filtrate is distilled as above and its color value determined. The nicotine content of blood is the difference between the two color values. A series of control experiments with nicotine added to blood gave results between 90 and 100% of the amounts added. The sensitivity permits the estimation of 1 microgram nicotine in 20 ml. blood. This method is suitable for the study of nicotine levels in the blood of smokers. The nature of the "blank material" has not been determined. Its magnitude is not influenced by nicotinic acid or trigonelline.

**Some crystalline peptides isolated from enzymic digests of insulin and their relationship to streptogenin** D. W. WOOLLEY *The Laboratories of The Rockefeller Institute for Medical Research* Because the amino group of streptogenin constitutes some of the free amino groups of insulin, the conversion of the protein to dinitrophenyl (DNP)-insulin, and subsequent digestion with proteolytic enzymes, should yield the growth factor in the form of DNP-streptogenin. This latter is colored yellow, and rendered distinct from most other products of digestion in that it is soluble in organic solvents. It may be differentiated from yellow impurities by the occurrence in it of glutamic acid.

Pancreatin digests of DNP-insulin, and of the DNP derivative of that portion of oxidized insulin which is soluble in water at pH 6, have been frac-

tionated by a series of countercurrent distributions between pairs of immiscible solvents, and several crystalline DNP peptides have been isolated. Aside from the yellow end group of DNP glycine contained in them they yielded the following amino acids when hydrolyzed: (1) glutamic acid, (2) glutamic acid, serine, threonine, valine and leucine, (3) same as (2) plus aspartic acid, (4) glutamic acid, serine, tyrosine, and leucine, (5) same as (3) plus isoleucine and cysteine acid (from cystine). The regular increase in complexity of these substances suggested that the smaller compounds were split products of the larger. Their composition affords an insight into the structure of a portion of the insulin molecule. Furthermore, peptides (2) and (3) were isolated from digests of DNP trypsinogen, from which fact it may be concluded that a sizable piece of the two proteins is identical. Finally, these observations may be of aid in the elucidation of the structure of streptogenin.

The application of paper chromatography to the study of amino aciduria in patients with liver disease. N. F. YOUNG and F. HOMBURGER (Introduced by T. F. GALLAGHER). The method of paper chromatography developed by Martin and

associates has made possible the separation and visualization of amino acids in complex mixtures. In this study this technique has been applied to the urines of patients with liver disease. Both hydrolyzed and unhydrolyzed urines from patients with infectious jaundice, homologous serum jaundice and hepatolenticular degeneration were chromatographed. The patterns so obtained made possible the estimation of the relative amount of each amino acid present. Total  $\alpha$  amino acid nitrogen excretion was determined on each sample by the ninhydrin reaction. The increase in  $\alpha$  amino acid nitrogen on hydrolysis is reflected in the chromatograms by increased amounts of some amino acids and the appearance of amino acids not found in unhydrolyzed urines. The results obtained will be compared to those obtained on normal urine. Although the degree and pattern of amino aciduria were found to vary greatly they did not closely correspond to the clinical changes observed. Neither was the severity of the liver disorder as measured by standard liver function tests found to parallel the extensivity of amino aciduria. The significance of the results will be discussed with respect to dietary and renal factors as well as liver function.

## AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC

### THIRTY-EIGHTH ANNUAL MEETING

Atlantic City, New Jersey, March 16, 16, 17, 18, 19, 1948

(For possible corrections in any of the following abstracts see the next issue)

Effects of amphetamine, dihydroergotamine, and methadon on human cerebral blood flow and oxygen uptake. BENEDICT E. ABREU and (by invitation) GRANT W. LIDDLE, Arthur L. Burks, ALEXANDER SIMON, VIOLETTE SUTHERLAND and GILBERT S. GORDAN. *Divisions of Pharmacology and Experimental Therapeutics, and Psychiatry, University of California Medical School, San Francisco*. Cerebral blood flow and cerebral oxygen consumption were determined by the Kety-Schmidt nitrous oxide technique on a series of unanesthetized humans before and after administration of amphetamine, dihydroergotamine and methadon. Following the intravenous administration of amphetamine sulfate 20 mgm, mean arterial pressures increased approximately 30%, and cerebral blood flow and cerebral oxygen consumption were decreased by approximately 20% and 15% respectively. Dihydroergotamine 1 mgm, intramuscularly, had no significant overall effect on arterial pressure, cerebral blood flow or oxygen utilization.

Amidone 5 mgm, intramuscularly did not produce conclusive effects.

| Subject                    | Blood flow (ml /100 gm brain/min) |            | Oxygen consumption (ml O <sub>2</sub> /100 gm brain/min) |            |
|----------------------------|-----------------------------------|------------|--|------------|
|                            | Control                           | After drug | Control  | After drug |
| <b>Amphetamine sulfate</b> |                                   |            |  |            |
| 1                          | 70                                | 54         | 4.3  | 3.6        |
| 2                          | 36                                | 31         | 2.7  | 2.3        |
| 3                          | 70                                | 58         | 4.1  | 3.5        |
| <b>Dihydroergotamine</b>   |                                   |            |  |            |
| 4                          | 44                                | 42         | 3.2  | 3.2        |
| 5                          | 67                                | 62         | 3.4  | 3.7        |
| 6                          | 70                                | 68         | 3.5  | 3.7        |
| <b>Methadon HCl</b>        |                                   |            |  |            |
| 7                          | 57                                | 70         | 3.2  | 4.1        |
| 8                          | 41                                | 38         | 3.0  | 2.4        |
| 9                          | 71                                | 69         | 4.2  | 4.3        |

**Comparative effects of sympathomimetic amines on the vasomotor resistance of the kidney, mesentery and leg** RAYMOND P. AHLQUIST *Department of Pharmacology, University of Georgia School of Medicine, Augusta* Five sympathomimetic amines, closely related structurally to epinephrine, have been compared as to their effects on the vasomotor resistance (VR) of the kidney, mesentery and hind leg of the anesthetized, heparinized dog. VR equals the arterial pressure (P), in mm Hg, minus 20, divided by the arterial flow (F), in cc per min. P minus 20 was used since the relationship between pressure and flow is markedly altered when P is lower than 20 mm Hg, due in part to the presence of the cellular elements in the blood. P was recorded by a Hamilton manometer, and F by a Shipley optical recording rotameter. Equimolar concentrations of the following racemic hydrochlorides were injected intra arterially: I, arterenol, II, cobefrine, III, epinephrine, IV, 3,4-dihydroxyephedrine, and, V, N-isopropyl arterenol. The results indicate that the vascular beds differ greatly in their sensitivity to sympathomimetic constrictors and dilators. In the kidney, III increases the VR the most, and IV and V have practically no effect. In the leg, however, I increases VR the most, and IV and V decrease the VR markedly. II is always less active than I in increasing the VR. The mesentery exhibits intermediate responses. The results also show that while epinephrine is the most active vasoconstrictor it is not the most active pressor agent, because it also possesses marked dilator activity which tends to diminish its pressor effect.

**The effect of rutin on oxygen toxicity in rats** SHANNON C. ALLEN and TALBOT G. MORTAROTTI (by invitation) *From the Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Albany, California*. It has long been known that high  $O_2$  tensions are toxic and they have been shown by other investigators to inhibit certain of the enzyme systems involved in cellular metabolism. On the theory that such inhibition might be reversed by a physiologic antioxidant Puig Muset and Valdecasas (1946) exposed rats to 100%  $O_2$  after pretreatment with vitamin P (eriodictyol) and found that it reduced the toxic effects. We have attempted to repeat their experiments using rutin and quercetin as vitamin P substances. Rutin, given in a single dose just before the experiment, had no effect on survival rate, weight loss or gross pathology of rats. When given in the diet and/or drinking water available throughout exposure to  $O_2$ , or in daily subcutaneous injections for 10 days previous to exposure, rutin and quercetin caused earlier deaths and fewer survivals. The most striking characteristic of the gross pathology of rats dying during exposure to  $O_2$  is the marked

hydrothorax and resulting lung collapse. There is a considerable body of experimental support for the theory that rutin exerts its physiological effects through the protection of circulating epinephrine and it has been shown by others that epinephrine has a dilator effect on pulmonary capillaries and enhances the susceptibility of young rats to  $O_2$  poisoning. This effect of rutin is further substantiated by the observations of R. H. Wilson of this laboratory that rats treated with rutin are more susceptible than controls to death from pleural edema following thiourea administration.

**Mode of action of antihistaminic agents** LILLIAN ALONSO (by invitation), MAXINE ADAMS (by invitation), LOUISE GODDARD (by invitation), MARION JAEGER (by invitation), and J. T. LITCHFIELD, JR. *Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut*. Four antihistaminic agents, Pyribenzamine, Neo-Integan, Chlorothene, and Benadryl, were studied for mode of action on atropinized guinea pig ileum. The concentrations of the antagonists used in these experiments ranged from 0.0001 micrograms/100 ml to 10 micrograms/100 ml of bath fluid, and those of histamine diphosphate from 0.3 micrograms/100 ml to 5250 micrograms/100 ml of bath fluid. For each antagonist the complete experiment was obtained on the same muscle strip, and a total of seventeen different experiments were carried out using the four different antagonists. The results of these studies indicate that the law of mass action is obeyed over a thousand fold concentration range of both histamine and antagonist. These results are in agreement with the theory that histamine and the above antagonists compete for the same cellular receptors.

**The effect of rutin and quercetin on scorbutic guinea pigs** ANTHONY M. AMBROSE *From the Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Albany 6, California*. Studies suggesting the use of flavone glycosides in the treatment of hemorrhagic manifestations of scurvy have been summarized (Nutritional Reviews 1943 and 1944). Evidence is based upon observations that foods rich in natural vitamin C are more effective than equivalent amounts of synthetic ascorbic acid in maintaining the integrity of the capillary wall. Studies have been undertaken to demonstrate the effect of rutin and quercetin in supplementing the action of subminimal doses of ascorbic acid in guinea pigs on a scorbutogenic diet. Young guinea pigs were placed on a scorbutogenic diet (Arch. Biochem., 12: 375, 1947), to which was added Brewer's yeast to make 1%. Supplements of cod liver oil, 1 ml, were fed per os every 5 days. Other supplements, rutin 100 mgm, quercetin 100 mgm, (contained in  $\frac{1}{4}$  ml of propylene glycol), or as

corbic acid 0.2 or 0.1 mgm (contained in  $\frac{1}{2}$  ml of water) were fed daily during the course of the experiment. Guinea pigs on the scorbutogenic diet when fed rutin or quercetin alone showed no improvement. However, when these same supplements were fed in conjunction with 0.2 or 0.1 mgm ascorbic acid mortality, compared with ascorbic acid controls, was reduced and the general appearance of the animals improved. Tentatively it may be concluded that rutin or quercetin both appear to have a sparing action on subminimal doses of ascorbic acid in the scorbutic guinea pig. Quercetin appears to be more effective than rutin, but this may be explained by the difference in molecular weight of the two substances.

The effect of rutin on blood pressure in dogs and rabbits. **ARTHUR M. AMBROSE** From the Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Albany 6, California. The effect of rutin on blood pressure in experimental animals has been variously reported. Fukuda (Arch. exp. Path. pharmacol., 164: 685, 1932) has reported a pressor effect in rabbits and frogs, while Czimmer (ibid. 183: 587, 1936) has observed no effect on blood pressure in cats. In conjunction with studies on capillary permeability the effect of rutin on blood pressure has been studied in dogs, under intravenous barbital anesthesia, and in rabbits, under oral urethane anesthesia. In 5 dogs in which rutin was administered intravenously in doses of 5, 20, or 100 mgm/kgm the effect on blood pressure was invariably depressor. The fall in blood pressure was in the order of 70 mm Hg and usually returned to normal within 3 minutes. The size of the dose of rutin had no particular influence on the magnitude of the fall in pressure, but the time of restoration to normal pressure increased with the dosage. In 6 rabbits, in which 100 mg/kgm was injected intravenously, five showed a fall in blood pressure similar to that observed in dogs, in two of which the blood pressure returned to within 10 mm of the normal value and in three the blood pressure rose to about 20 mm above the normal after the initial fall, with a gradual return to normal. In one rabbit the effect was initially pressor, followed by a depressor effect, and return to approximately normal within  $\frac{1}{2}$  hr after administration.

**Parasiticidal activity of thioarsenites in man.** **HAMILTON H. ANDERSON**, **HERBERT G. JOHNSTONE** (by invitation), and **A. PEÑA CHAVARRIA** (by invitation). University of California Medical School, San Francisco, California, and Hospital San Juan de Dios, San Jose de Costa Rica, A. C. The dithiocarbonylmethyl and dithiocarbonylphenyl derivatives of p-carbamidophenyl arsenous oxide have exhibited activity against *E. histolytica* in vitro and in infected macaques (J. Pharmacol.,

91: 112, 1947). During the course of field trials in human amebiasis, the thioarsenites were employed against other human parasites. The adult (65 kilo) dose was 200 mgm given orally thrice daily for 10 days. There were 12 persons harboring *Strongyloides stercoralis*, only one of whom was cleared, 2 with *Balantidium coli*, both cleared, 1 with *Fasciola hepatica*, with uncertain results, and 2 with *Leishmania tropica* and 3 with *Treponema pertenue*, none of whom were cleared. In each case tests of urine, blood, liver and kidney function showed no change during or after treatment, except increases in hemoglobin levels and body weight due to improved nutrition and bed rest. No evidences of drug intolerance developed except nausea in 2 which was controlled by salol coating of tablets.

The action of methadon upon the respiration of rat diaphragm and liver and kidney cortex slices. **ARTHUR L. BACHELOR** and **HENRY W. ELLIOTT** (introduced by **BENEDICT E. ABREU**). Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco. The action of methadon upon the respiration of rat cerebral cortex slices has been previously reported (Elliott, Warrens, and James, J. Pharmacol., 91: 98, 1947). The studies have been extended to include other tissues using methods described in the reference cited. Pieces of young rat diaphragm and liver and kidney cortex slices were subjected to concentrations of methadon which (a) stimulated (0.0005M), (b) produced a delayed inhibition (0.001M) and (c) strongly inhibited (0.002M) brain slice respiration. The results are summarized in the following table.

| Tissue    | Concentration |          |           |          |          |          |
|-----------|---------------|----------|-----------|----------|----------|----------|
|           | 0.0005M       |          | 0.001M    |          | 0.002M   |          |
|           | E             | T        | E         | T        | E        | T        |
|           | %             | min      | %         | min      | %        | min      |
| Diaphragm | 153<br>110    | 30<br>90 | 120<br>57 | 15<br>90 | 134<br>5 | 15<br>90 |
| Kidney    | 82            | 90       | 34        | 90       | 16       | 90       |
| Liver     | 56            | 90       | 22        | 90       | 7        | 90       |

E =  $Q_0$  as per cent of control      T = Time of maximum effect

The dual effect of methadon found for brain slices was shown to an even greater degree by diaphragm. Both 0.001 and 0.002M methadon produced a delayed inhibition of diaphragm respiration but all concentrations increased the oxygen consumption of diaphragm more than they stimulated brain respiration. There was no evidence of stimulation of liver or kidney cortex respiration at any time after addition of the drug. The selective stimula-

tion of brain and diaphragm respiration is in marked contrast to the action of dinitrophenol which has been shown to increase the oxygen consumption of all tissues included in these studies

The maintenance of mean pulmonary arterial pressure after extreme right ventricular damage in the dog AUGUSTUS C P BARNES (introduced by CHARLES F MORGAN) *Dept of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D C* Severe right ventricular damage was produced in barbitalized and heparinized dogs by repeated charring with a soldering iron as described by I Starr (*Am Heart J* 26: 291, 1943) Simultaneous mercury manometric recordings of mean systemic and pulmonary arterial pressure fluctuations were made from direct cannulations of the carotid and a right pulmonary arterial stem branch respectively Peripheral venous pressure was determined directly by cannulation of the femoral vein The degree of right ventricular damage produced was ascertained at the termination of each experiment by grossly estimating the area of epicardial surface charred and the depth of charring, thus over 75 per cent necrosis of the right ventricular muscle could be obtained No gross pulmonary edema or congestion was noted at this time The results of these preliminary studies on nine dogs showed that following the production of severe right ventricular damage the mean pulmonary and systemic arterial pressures had not changed significantly from pressures recorded prior to the damage Similarly the peripheral venous blood pressure also remained fairly constant as previously reported by Starr It was further observed that coincident with left ventricular systole the right ventricle showed passive enlargement and failure of active contraction In two of these experiments, subsequently produced left ventricular damage resulted in a simultaneous decline in the pressures of both the pulmonary and systemic arterial systems The relationship of these experiments to the dynamics of right ventricular failure is yet to be considered

The adrenergic properties of antihistaminic drugs determined by electrical measurements T C BARNES *Dept of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia* In the "oil-cell" method (Barnes and Beutner, *Science* 104: 569, 1946) drugs are placed at the interface between saline and an oil layer and the resulting phase-boundary potential is measured For example the negative potential produced by acetylcholine and prostigmine (Barnes, *Arch internat Pharmacodynamie* 73: 386, 1947) may explain their stimulating action The oil (representing the lipid layer on the living cell) is held in a cup on a U-shaped glass tube immersed in saline To avoid possible short circuits along the glass surface the drug solution and the saline on

the opposite side of the oil layer may be introduced by two separating funnels dipping into a vessel of oil The triglyceride oils have the special property of dissolving sympathomimetic drugs and thereby establishing a negative phase boundary potential (Barnes, *Anat Rec* 96: 87, 1946, Seventeenth International Physiological Congress 60: 1947) Acetylcholine has no effect on these oils It was surprising to find that benadryl and pyribenzamine behave like sympathomimetic drugs on the triglyceride oils 0.05% benadryl produced 40 mv negative on triacetin in saline 0.05% pyribenzamine produced 15 mv negative on triacetin Histamine has no electrical effect on triacetin The results emphasize the possible adrenergic properties of these drugs According to Sherrod pyribenzamine potentiates the pressor action of epinephrine Loew has reported experimental bronchodilatation with benadryl

Phase-boundary potentials of some analogs of prostigmine. T C BARNES *Dept of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia* Prostigmine has outstanding electrical activity *in vitro* (Barnes, *Anatomical Record* 96: 87, 1946, *Fed Proc* 6: 74, 1947 *Arch internat Pharmacodynamie* 73: 386, 1947) The phase-boundary potentials of several analogs of prostigmine are shown in the table Drugs were added to

| Prep    | 0.001 M on cresol | 0.001 M on guaiacol | 1 mg on oleate-nitrobenz | 10 mg on oleate-nitrobenz |
|---------|-------------------|---------------------|--------------------------|---------------------------|
|         | mv                | mv                  | mv                       | mv                        |
| A. Ch   | 44                | 23                  | 75                       | 110                       |
| Prostig | 54                | 60                  | 96                       | 130                       |
| Nu 613  | 50                | 60                  | 90                       | 145                       |
| Nu 1250 | 40                | 57                  | 90                       | 150                       |
| Nu 1197 | 45                | 60                  | 41                       | 100                       |
| Nu 1208 | 40                | 60                  | 47                       | 122                       |
| Nu 1243 | 34                | 60                  | 71                       | 123                       |
| Nu 1249 | 47                | 60                  | 69                       | 130                       |
| Nu 1173 | 36                | 51                  | 60                       | 120                       |

200 cc of aqueous medium containing the oil cup (Barnes and Beutner, *Science* 104: 569, 1946) In the first column are numbers of the compounds For chemical and biological data see Aeschlimann and Stempel in the *Barell Volume*, Basle, p 306, 1946 The negative phase-boundary potentials in millivolts are recorded in the next four columns Columns two and three are potentials produced by 0.001 molar concentrations in saline in contact with cresol and with guaiacol The last two columns list potentials of one milligram and of ten milligrams at the interface between nitrobenzene and 200 cc of equal parts saline and 0.1% sodium oleate The data indicate the pronounced electrogenic action of all prostigmine analogs Further studies may explain individual differences

The toxicity of polyallyl alcohol T R W

BARNES (introduced by CHARLES H HINI) *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco* When administered intragastrically as a 50% aqueous solution, polyallyl alcohol produced no gross toxic effect in mice or rats which received as much as 27 gm/kg. Single administrations were made to 30 animals of each species, which were observed over 10 days. Skin irritation was tested on rabbits according to the method of Draize, et al (J Pharmacol, 82 377, 1941). A saturated solution of polyallyl alcohol in ethanol was applied to the shaved dorsal surface of albino rabbits. It produced neither irritation nor systemic effects in amounts up to 1.0 gm/kg. Only a slight conjunctivitis resulted after instillation of a 50% aqueous solution of polyallyl alcohol into the eyes of rabbits. On sacrifice of all groups at the end of 10 days, neither gross nor microscopic pathologic changes were observed. Polyallyl alcohol is a polymer of allyl alcohol, and has a molecular weight of 550 and an average degree of polymerization of 9.5. Polymerization apparently abolishes the toxic properties of the parent allyl compound.

**Toxicological and pharmacological data concerning 2-benzyl-imidazoline hydrochloride (Priscol)** W BARRETT, A CAMERON, N HANSEN, E HERROLD, A MACKENZIE, B RICHARDS, F ROTH, J SMITH (all by invitation), (introduced by F F YONKMAN) *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey* A continuing study of Priscol's action has yielded data which have confirmed and extended the original work of Meier and his associates (R. Meier and R. Mueller, *Schw. med. Wschr.* 69 1271, 1939; R. Meier and R. Mueller, *ibid.* 71 1206, 1941). Toxicity LD<sub>50</sub> white rats IV—85-95 mg/kg, IP 100-125 mg/kg, and po 1200-1400 mg/kg. Dogs have tolerated orally up to 140 mg/kg with subsequent recovery. Doses of 2.5, 5.0 and 10 mg/kg have been administered per os 5 days a week for 60 days to dogs without revealing any significant manifestations of toxicity as determined by weight curves and hematological studies. The final results of this test, which is to continue another 30 days, will be reported. Priscol has positive, inotropic action upon isolated, perfused hearts of the guinea pig and cat. Doses from 5.0 to 20 mg stimulate the former without concomitant or subsequent evidences of inhibition. Doses from 5.0 to 200 mg stimulate the feline heart with a slight, secondary inhibition after the highest. In the canine heart-lung preparation doses of 50 to 100 mg exerted a mild, positive inotropic effect, lower doses exhibited no action, and doses totaling 200 mg caused no evidences of toxicity. Warburg studies have indicated that concentrations less than 5.0 mg/cc do not inhibit tissue

respiration. The tissues studied have included renal, hepatic and cardiac slices from the rat and also feline cardiac slices. The minimal inhibitory doses varied from 5.0 to 20 mg/ml. The kidney of the rat was most sensitive, and the feline heart least. The drug has revealed mild oxytocic and smooth muscle stimulating properties.

**Effect of ion-deficient Locke's solution on guinea pig ileal responses to histamine, acetylcholine, electrical stimulation** W BARRETT (by invitation), B CRAVER *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey* Duplicative control responses to the stimulants were elicited from the ileal strip perfused with normal Locke's solution, after which perfusion with deficient Locke's solution was initiated and the responses followed until constant. Then the effects of adding variable increments of the missing salt to the bath were determined before determining the effects of reperfusion with normal Locke's solution. The deficient solutions lacked calcium, magnesium or potassium alone or by pairs. The effects of each deficiency were reversible since sufficient reperfusion with normal Locke's solution always restored responses. The following table summarizes the results.

| Missing ions | Effect on responses | Action of increments             | Re-perfusion     |
|--------------|---------------------|----------------------------------|------------------|
| Mg           | increase            | low doses—0, 1.0 mg/cc—decrease  | normal responses |
| Ca           | absence             | normal responses                 | normal responses |
| K            | marked decrease     | slight increase                  | normal responses |
| Ca Mg        | absence             | Mg—O Ca—normal                   | normal responses |
| Ca K         | absence             | K—O Ca—increase<br>K + Ca—normal | normal responses |
| Mg K         | absence             | Mg—O K—increase<br>Mg + K—normal | normal responses |

The last 5 solutions caused loss of responsiveness to electrical stimulation, this was always the first stimulant to become ineffective. Intestine sensitized to horse serum and perfused with Ca-deficient solutions did not respond to the antigen until 40-80 mg/cc of Ca had been added to bath.

**Response of experimental lymphoma to certain organic sulfides, sulfones and chlorides**, ALLAN D BASS *Pharmacology Department, Syracuse University* It has been previously shown that bis-, (chloroethyl)sulfide is a lymphocyte destroying agent. Certain organic sulfides, sulfones and chlorides have been selected for this study in the hope of finding a less toxic agent with lympholytic action. Mouse tumor 6C3HED was used. The chemical used was administered intraperitoneally

to approximately ten C3H mice bearing the transplanted lymphosarcoma. Growth curves were plotted for each substance [tested]. Propylene glycol was used as a solvent or diluent where possible, but in a few instances a propylene glycol ethyl alcohol mixture was required, and in a few instances a coconut oil emulsion was employed. Toxicities were first determined in Rockland white mice. Roughly one fourth of this dose was found to be near the minimum lethal dose for the tumor bearing C3H mice. Of the compounds studied acetodichlorohydrin proved the most effective agent. Certain problems arising from the use of solvents or diluents producing non specific tissue damage will be discussed. These factors were found to be important because of the relationship of non specific tissue damage to lymphoid tumor regression.

**The effectiveness and toxicity of methadon**  
 ROBERT C. BATTERMAN and ABRAHAM M. OSHLIG (by invitation) *Department of Therapeutics, New York University College of Medicine*. In a group of 150 hospitalized and 30 ambulatory patients, methadon in doses of 2.5 to 20.0 mg. in a single dose or in repeated doses several times daily controlled pain in only 40 per cent of the trials when administered orally and 76 per cent of the trials when used parenterally. Toxic symptoms such as dizziness, nausea, vomiting, weakness and drowsiness were frequently noted occurring in hospitalized patients in 23 and 40 per cent for parenteral and oral administration respectively and 80 per cent for ambulatory patients. When patients above the age

50 receive the drug repeatedly for several days, severe mental confusion and toxic psychosis was noted in 30 per cent of the trials. Methadon is not a satisfactory analgesic. The high incidence of untoward reactions noted after chronic administration and in ambulatory patients limit its clinical usefulness.

**Infectivity of sporozoites of Plasmodium Cathemerium 3H2 exposed in vitro to hen and canary bloods**  
 HARRY BECKMAN *Marquette University School of Medicine*. Mosquitoes carrying the sporozoites of *P. cathemerium* 3H2 were ground in Locke's solution and added simultaneously to freshly drawn canary (susceptible) and hen (susceptible) bloods. The two blood sporozoite mixtures were incubated with gentle agitation at 41.5°C., and at thirty-minute intervals through two and a half hours increments representing one mosquito each were withdrawn and injected intramuscularly into canaries, the peripheral blood of these birds being subsequently examined from the fifth to the sixteenth days, inclusive. Birds failing to become positive were challenged by mosquito bite. Trials were run at sufficient intervals that at least one complete three week sporozoite-to-sporozoite cycle lay between. Of the total of eighty

canaries injected with sporozoites previously incubated in canary blood, seventy-eight (97.5%) showed patent blood infections, of the seventy-six canaries injected with sporozoites previously incubated in hen blood, two (2.6%) showed patent blood infections. All birds failing to become positive were subsequently successfully infected by mosquito bite. It would appear that the blood of the hen contains a factor capable of preventing patency of *P. cathemerium* 3H2 in canaries injected with sporozoites previously incubated in it.

**Studies on a heparin-like compound, algarin**  
 ALBERT J. BÉGANI (by invitation) and JOSEPH SLIFTLER *Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.* Algarin is a sodium salt of an alginic acid disulfuric ester containing 15-17% sulfur. In common with other polysaccharide-polysulfuric acid esters it is a powerful anticoagulant *in vivo* as well as *in vitro*. It differs from the heretofore described substances of this class in its relatively low toxicity and failure to produce hemorrhage of the viscera. Large doses do not affect the blood pressure when injected intravenously. In this study data is presented comparing Heparin and Algarin by intravenous injection in rabbits, cats, and dogs. Algarin appears to be  $\frac{1}{2}$  as active as Heparin depending on species employed. When these ratios are employed the intensity and duration of effect are practically identical for Algarin and Heparin. Algarin is not inactivated by the liver. It is antidoted by intravenous injections of protamine sulfate or toluidine blue.

**Prolonged depressor effect of water soluble organic nitrates**  
 JOSEPH G. BIRD (introduced by JOHN C. KRANTZ, JR.) *Department of Pharmacology, Univ. of Maryland, School of Medicine, Baltimore*. It has been observed that no reduction of blood pressure in the dog is produced by the intravenous injection of the sodium salt of glycollic acid nitrate in doses equal to effective amounts of glyceryl trimnitrate. However, larger doses evoke a depressor response more prolonged than responses to glyceryl and erythritol nitrates. Of particular interest is the insensitivity of the lowered arterial pressure to repetition of the dose of glycollic nitrate although the foregoing nitrates still produce their transient depressor action. Malic and tartaric acids have been nitrated and are being studied. Water solubility appears to be an important factor in the action of organic nitrates.

**An examination of potentiating action of aspirin upon codeine in raising the pain threshold**  
 D. D. BONNYCASTLE and JACOB MOLLAND (by invitation) *Departments of Pharmacology, Yale School of Medicine, and University of Oslo, Oslo, Norway*. In this investigation a modification of the D'Amour-Smith radiant heat apparatus has been employed. Rats were used as the test animals,



and in carrying out this experiment special consideration has been given to certain factors which we have shown to be important in this type of work, viz

(i) Training periods are necessary for the animals before use

(ii) A daily cut off time of stimulation must be established to avoid injury which might impair the animals' usefulness in the carrying out of subsequent tests

(iii) An adequate statistical design should be used for this type of work

Acetylsalicylic acid in dose levels that have little or no effect upon the pain threshold have been used with graded doses of codeine sulphate. The dose levels of codeine sulphate range from those that are without effect to ones having a definite action in raising the pain threshold

Re-evaluation of the effectiveness of metrazol as an analeptic agent in barbiturate depression, WALTER M. BOOKER, DAVID M. FRENCH, PEDRO A. MOLANO, and CECIL RHODES (introduced by A. H. MALONEY) *From the Department of Pharmacology, College of Medicine, Howard University, Washington, D. C.* A growing controversy is more and more imminent as to the effectiveness of metrazol in overcoming barbiturate depression. Interesting divergent pieces of experimental evidence have been published. The increasing widespread use of pentothal sodium as an anesthetic agent has accentuated and sharpened the opposing views. We present evidence showing that metrazol is effective in stimulating respiration and even arousing animals depressed by pentothal sodium. Groups of dogs have been anesthetized by administering pentothal sodium intravenously at 20, 30, 40 and 50 mgm./kgm. body weight, and the mgm./kgm. ratio of metrazol necessary to restore normal respiration and arouse the animals at each level of barbiturate administration was determined. Figures varying from 125 mgm./kgm. body weight to 500 mgm./kgm. body weight of metrazol are presented, in relation to the varying dosage levels of pentothal administered. In a number of animals blood pressure tracings, expired air values, and arterial oxygen saturation values have been obtained. Our evidence seems to show that although respiratory rate may be increased in most instances where high doses of metrazol are used, the depth of respiration is of much more importance in improving the condition of the animal. This is shown by oxygen saturation of arterial blood and by the state of the blood pressure.

The mechanism of the extraction of bromsulphalein from blood plasma by the liver. RALPH W. BRAUER and RITA L. PESSOTTI (introduced by CHAPMAN REYNOLDS) *Department of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State University, New Or-*

*leans, La.* Bromsulphalein (BSP) forms compounds with various plasma proteins. With bovine plasma albumin (crystalline) (CBPA) the reaction involves one molecule of dye and one of protein, and is reversible (dissociation constant  $7 \times 10^{-4}$ ). In human plasma most of the BSP is associated with the true albumin fraction. Liver homogenates likewise form non dialyzable complexes with the dye. Rat liver slices incubated with low concentrations of BSP in Krebs solution take up insignificant amounts of the dye. With increasing concentrations of BSP dye uptake by the tissue occurs in such fashion that a constant BSP concentration remains in the supernatant despite considerable further increase in the initial dye concentration. Once the storage capacity of the liver slices is exceeded, all of the excess dye appears in the supernatant. The addition of CBPA to the Krebs solution reduces the fixation of BSP by the liver. In perfused rat livers the percent dye extraction from Locke's solution is almost independent of the initial concentration. Increased perfusion rates or the addition of CBPA to the perfusion fluid reduces the proportion of BSP extracted. Continuous iv BSP infusion (normal dogs) produces steady plasma and bile levels, biliary excretion at 70-100% infusion rate. In each animal a constant proportion (60-85%) of the dye is removed from blood passing through the liver, regardless of the systemic plasma level. These and related results obtained under various abnormal conditions indicate a minimal participation of the RC system in the extraction of BSP from plasma. Instead, an exchange of protein bound dye between plasma and hepatic cells is proposed as the basic mechanism.

The physiological disposition of acetophenetidin (p-ethoxyacetanilide) in man. BERNARD B. BRODIE and JULIUS AXELROD (by invitation) *Department of Biochemistry, New York University College of Medicine, and New York University Research Service, Goldwater Memorial Hospital, and Laboratory of Industrial Hygiene, N. Y., N. Y.* Previous work concerning the metabolism of acetanilide (unpublished) has shown that acetanilide deacetylates in small part in the body to aniline, the precursor of the substance which oxidizes hemoglobin to methemoglobin, and, in large part, to N-acetyl p-aminophenol, an active analgesic. The metabolism of acetophenetidin has also been studied as part of a program concerning the fate of aniline derivatives in the body. The route of metabolism of acetophenetidin was shown to be as follows: A minor fraction of the drug deacetylates to form p-phenetidin (p-ethoxyaniline). This compound was shown to be the precursor of the substance which oxidizes hemoglobin to methemoglobin. The major fraction of the drug deethylates rapidly to form N-acetyl p-aminophenol.

The short life of the parent drug in the body and its rapid alteration to N acetyl p aminophenol leads to the conclusion that the analgesic activity of acetophenetidin is due to this derived product. Preliminary work with N acetyl p aminophenol indicates that it is an effective analgesic and may be given frequently in relatively large doses without producing methemoglobin.

#### Quantitative epinephrine pressor effects

ROBERT V BROWN *Division of Pharmacology, University of Tennessee, Memphis* Past experiments on the pressor responses to epinephrine have produced dose effect curves defined as linear, logarithmic, hyperbolic, or exponential. The effects were measured as rises in blood pressure by some, as peak blood pressures by others. Hjort, et al, (*J Pharmacol & Exper Therap* 71 105, 1941) fit their data best with the equation  $\log y = b \log x + \log a$ , where  $y$  is the increase in blood pressure and  $x$  is the dose of epinephrine. Their data were averaged and a single curve was published. In a series of experiments in dogs, pressor effects were measured both as blood pressure increases and as peak blood pressures. Both sets of effects follow the log dose-log effect regression line, but the log dose-log peak equation, determined by the least squares method, yields a much better fit to the experimental data than the log dose-log increase equation. When the dose is in  $\mu\text{gm}/\text{kgm}$ , the constant,  $\log a$ , is the intercept where  $x = 1$  or  $\log x = 0$ . The constant,  $\log b$ , is the slope of the regression line. Both constants are measures of the sensitivity of any particular animal to the pressor effects of epinephrine. They vary from animal to animal and each has two values, depending on whether increases in blood pressure or peak blood pressures are used. It is possible to fit this equation to various already published data. The previously proposed equations diverge continuously from the experimental data as the lower range of dosage is traversed. The most accurate dose effect curve, where  $Y$  is the peak blood pressure and  $x$  is in  $\mu\text{gm}/\text{kgm}$  is  $Y = Ax^n$  or  $\log Y = B \log x + \log A$ .

**On the mechanism of the antitussive action of amidone** MARION BUELL (by invitation), JOAN E COPELAND (by invitation) and ELDON M BOYD *Dept of Pharmacology, Queen's Univ, Kingston, Canada* The morphine-like action of amidone has naturally suggested its trial in the symptomatic therapy of cough due to the common cold and a number of other conditions. If it be accepted that amidone is an effective antitussive drug, the question arises as to whether it acts by increasing the secretion of bronchial or respiratory tract fluid (R T F), or by centrally depressing the cough reflex, or in some other manner. The first mode of action noted has been investigated using a modification of the technique of Peirv and Boyd (*J Pharmacol & Exper Therap*, 73 65, 1941). Ex-

periments were performed upon several species of animals, the drug being given both orally and parenterally in a series of doses ranging from 0.1 to 10 mgm per kilo body weight. In no species could there be demonstrated a statistically significant change in the volume output of R T F. In this negative pharmacodynamic action, amidone apparently acts like morphine which also has been shown to have no effect upon the volume output of R T F (Boyd and MacLachlin, *Canad Med Assoc J*, 50 338, 1944).

**Pharmacological studies of myanesin and curare** JOHN C BURKE (by invitation) and CHARLES R LINGGAR *Pharmacological Development Division, E R Squibb & Sons, New Brunswick, New Jersey* The pharmacological action of myanesin, 3-(o toloxy)-1,2 propanediol, has been compared with that of d-tubocurarine chloride in various animals. Myanesin produces muscular relaxation similar in appearance to that of curare. Degree of relaxation with both drugs is dependent upon dosage, and complete relaxation of postural muscles can be accomplished without paralysis of respiratory muscles. This paralysis can be used as the "head drop" end point in the assay or the comparison of such drugs. Onset of paralysis with myanesin is more gradual in response to dose than with curare. Partial paralysis by myanesin is characterized by incoordination, stupor and partial analgesia, while curare produces only simulated muscle weakness. Myanesin occasionally causes transient rigidity, frequently nystagmus at the peak of paralysis, and nausea and vomiting during recovery. These reactions are absent with curare. Myanesin shows a wider species difference in potency than curare but approximately the same therapeutic ratio. d-Tubocurarine chloride is 230 to 1,000 times as potent as myanesin intravenously in different species while the duration of action of the two drugs is similar. Myanesin exhibits some local anesthetic activity, curare none. Myanesin causes severe vasodepression at relaxing dosage, and severe cardiac depression or block at respiratory paralyzing dosage. It also causes significant hemolysis *in vivo* and *in vitro*. The paralyzing action of myanesin is due to depression of the central nervous system, reaching above the spinal level, and to some slight peripheral or myoneural depression, while that of curare is peripheral, being accomplished without significant vasomotor, cardiac or central nervous system depression.

**A comparison of the effects of ether and cyclopropane on renal function in man** BURNETT, CHARLES H. (by invitation), GORDON, ESTHER B. (by invitation), SHORTZ, GERALD (by invitation), COMPTON, DAVID W. (by invitation) and BLECHER, HENRY K. *Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital and the Department of Medicine, Massachusetts General Hospital* The effects of anesthesia

upon renal clearance of mannitol and sodium para-amino-hippurate in 8 patients under ether and 7 under cyclopropane have been compared. Only patients in good clinical condition with essentially normal renal function were chosen, and all studies were completed before surgical procedures were started. Anesthesia was maintained in the second plane of the third stage. Average observed changes are shown in the table. Individual protocols reveal

| Anesthesia   | No patients | Average changes (per cent) |                  |                                  |            |
|--------------|-------------|----------------------------|------------------|----------------------------------|------------|
|              |             | C <sub>M</sub>             | C <sub>PAH</sub> | C <sub>M</sub> /C <sub>PAH</sub> | Urine flow |
| Ether        | 8           | -21.2                      | -38.7            | +25                              | -45        |
| Cyclopropane | 7           | -32.5                      | -53.3            | +35                              | -42        |

some trends and differences not apparent from mean values. One subject showed no significant change in either mannitol or para-aminohippurate clearance during ether, in a few other ether subjects clearance of both substances following an initial drop soon after induction improved as anesthesia continued. This trend toward improvement was absent or much less marked in the cyclopropane group. Carbon dioxide contents of the serum fell slightly with ether and were unchanged with cyclopropane. In so far as could be determined, extrarenal factors were excluded or so minimized that the observed changes in glomerular filtration rate and effective renal plasma flow represent effects on the kidneys *per se* of these agents. The high filtration fractions suggest that the renal ischemia is at least partly due to efferent arteriolar constriction. Our data indicate that while qualitatively the effects of ether and cyclopropane on renal function are similar, quantitatively those of cyclopropane are more marked in reducing renal function.

Actions and minimally effective doses of 17 common drugs on perfused hearts of 5 species. A. CAMERON (by invitation), M. LASKE (by invitation), B. CRAVER, *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey*. Since a standard method for studying the responses to drugs of isolated, perfused hearts had become available (F. F. Anderson and B. N. Craver, *Fed Proc* 6:48, 1947), it seemed valuable to prepare a table recording the quantitative and qualitative differences in responses to common drugs of the hearts of the usual laboratory animals. Such a table as the following would not only be a convenience to those employing the Langendorff preparation, but might provide clues to modes of drug action and cardiac metabolism.

The use of ultraviolet rays in a screening test for some barbiturates and in reading Gutzzeit papers. W. J. R. CAMP, V. A. GANT (by invitation)

| Drug                       | Cat                    | Rabbit              | Guinea pig                  | Rat                         | Hamster               |
|----------------------------|------------------------|---------------------|-----------------------------|-----------------------------|-----------------------|
| Acetylcholine              | 1 0y<br>—              | 0 1y<br>+           | 0 005-<br>0 01y<br>±        | 0 005y<br>—                 | 0 005y<br>—           |
| Aminophylline              | 100-200y<br>+ —        | 100-200y<br>—       | 10-50y<br>+                 | 5 0y<br>—                   | 3 5y<br>—             |
| Antistine                  | 20-50y<br>—            | 20-50y<br>—         | 5 0y<br>—                   | 5-10y<br>—                  | 10y<br>—              |
| Atropine*                  | 20y<br>—               | 20-50y<br>—         | 10-20y<br>—                 | 1-2y<br>—                   | 0 5y<br>—             |
| Digifoline                 | 0 025-0 05<br>c u<br>+ | 0 05 c u<br>+       | 0 025 c u<br>+              | 0 05-0 1<br>c u<br>+        | 0 1 c u<br>+          |
| Doryl                      | 0 5y<br>—              | 0 25-0 5y<br>+      | 0 001-<br>0 005y<br>±       | 0 002y<br>—                 | 0 002y<br>—           |
| Ephedrine                  | 0 5-1 0y<br>+          | 4 0y<br>—           | 0 5y<br>+                   | 2 0y-<br>5 0y<br>+          | 1 0-2 0y<br>—         |
| Epinephrine                | 0 019y<br>+            | 0 01-<br>0 02y<br>+ | 0 01-<br>0 02y<br>+         | 0 01-<br>0 02y<br>+         | 0 02y<br>+            |
| Histamine                  | variable               | 0 2y<br>—           | 0 005-0 01<br>y<br>±        | 5 0y<br>—                   | 10-20y<br>±           |
| Mecholyl                   | 1 0y<br>—              | 0 5-1 0y<br>+       | 0 002-<br>0 005y<br>+       | 0 005y<br>—                 | 0 005y<br>—           |
| Papaverine                 | 50-100y<br>+           | 50-100y<br>—        | 2 0-5 0y<br>+               | 5-10y<br>+                  | 0 5y<br>—             |
| Pituitrin (S)              | variable               | 0 002 I U<br>+      | 0 002-<br>0 004<br>I U<br>+ | 0 001-<br>0 002<br>I U<br>+ | 0 01-0 02<br>I U<br>— |
| Priscol                    | 5-20y<br>+             | 200-300y<br>—       | 5 0y<br>+                   | 10y<br>—                    | 10-20y<br>—           |
| Privine                    | 20-50y<br>—            | 10y<br>—            | 0 5y<br>—                   | 1 0y<br>—                   | 0 5-1 0y<br>—         |
| Pyribenzamine              | 20-50y<br>—            | 50y<br>—            | 5 0y<br>—                   | 5-10y<br>—                  | 10y<br>—              |
| Tetraethylammonium bromide | variable               | variable            | 20y<br>—                    | 10y<br>—                    | 3 0-5 0y<br>—         |
| Trasentine                 | 20-100y<br>—           | 50-100y<br>—        | 5-10y<br>—                  | 3 0y<br>—                   | 5-10y<br>—            |

\* This dose mildly inhibits cardiac action, but lower doses (2-3y) block effective doses of Doryl and Mecholyl. + = stimulation — = inhibition

and JOHN F. POLLI (by invitation), *Dept of Toxicology and Pharmacology, Univ of Ill College of Medicine, Chicago 12*. When a large number of

toxicological analyses are to be made, any procedure which will expedite or simplify the procedures is of importance. The number of substances dispensed in colored capsules is rather limited, and from the viewpoint of toxicology really limited to a few very commonly used barbiturates. Occasionally one may find remnants of colored capsules in the stomach contents. The dyes used in the capsule are not sufficient to distinctly color the specimen but are in sufficient quantity to reveal a very definite fluorescence when exposed to filtered ultraviolet rays. While this procedure is not indicative of barbiturates, since other materials, e.g., Benzidyl, are dispensed in colored capsules, it is helpful in shortening chemical procedures when a history is wanting and the fluorescence is positive. Naturally the absence of a fluorescence should not exclude analysis for barbiturates. A second use for the ultraviolet lamp is for a more accurate measuring of the stain on Gutzzeit paper. A golden glow is seen to sharply limit the area of unchanged mercuric bromide. The strip is marked under the light and compared with standard measurements similarly made. We have found that strips negative to the eye show a definite fluorescence. While there is a slight increase in the sensitivity we feel the ease in accurately reading the strip is of more importance.

The acute toxic effects of dicumarol. ROSE MARIE CARLSON (by invitation) and LLOYD D. SEAGER, Department of Pharmacology, The Woman's Medical College of Pennsylvania. The observations of Rose, Harris and Chen, *Proc. Soc. Exp. Biol. and Med.*, 50: 228, 1942 on the toxic effects of dicumarol have been largely confirmed. In addition a direct toxic effect of the drug on the heart has been found. In the cat, rabbit, rat and mouse the intravenous or intraperitoneal injection of 100 to 300 mgs per kg produces death within a few hours. Death is preceded by convulsions. Animals biopsied during convulsions have invariably shown cardiac arrest usually involving both auricles and ventricles and often presenting fibrillation. Blood pressure tracings show cardiac acceleration with little change in pressure until the terminal abrupt fall. The frog also presents cardiac arrest after the lymph sac injection of 200 mgm per kg. It is suggested that the convulsions from large doses of dicumarol are asphyxial in nature and that the pulmonary edema frequently encountered is in part cardiac in origin.

The LD<sub>50</sub> of pentobarbital in nursed and unnursed newborn rats. EMMETT B. CARMICHAEL and WALTER H. JOHNSON (by invitation), Biochemistry Department, Medical College of Alabama, Birmingham. Young rats less than 24 hours old were used. All injections were made intraperitoneally and the animals were immediately placed in an incubator to avoid chilling. The stoppage of the heart beat was taken as the time of death. The

doses employed were 30, 40, and 50 mg/kg body weight. The rats were separated into three weight-groups: (1) 10-199 grams, (2) 50-599 grams, and (3) 600-750 grams. The nursed rats survived a larger dose than did those that did not nurse. The LD<sub>50</sub> for the nursed rats seems to be almost 10 mg/kg while the LD<sub>50</sub> for those that did not nurse was about 30 mg/kg.

A comparative study of cyclic and noncyclic hydrocarbons on cardiac automaticity. C. JELLEFF CARP and JOHN C. KRAVITZ, JR., Department of Pharmacology, Univ. of Maryland, School of Medicine, Baltimore, Muth, Hathaway and Orth (1937) showed that arrhythmias frequently occurred in the dog's heart under cyclopropane anesthesia when the automatic tissue of the heart was sensitized by intravenous injections of epinephrine. In this laboratory it has been shown that the *Macacus rhesus* monkey behaves in a similar manner and that cyclobutane anesthesia sensitizes the dog's heart to epinephrine similar to cyclopropane anesthesia. In the present study the effects of other hydrocarbons related to cyclobutane and cyclopropane have been investigated. Anesthesia in the dog under cyclobutane, cyclobutene, butane, isobutane and *cis-trans* 2-butene sensitizes the automatic tissue of the myocardium to epinephrine. Anesthesia under ethylene does not elicit this response. Cyclobutane and cyclobutene produce an excellent anesthesia in the dog, butane, isobutane and *cis-trans* 2-butene do not evoke satisfactory anesthesia in the dog.

The anaphylactic guinea pig trachea and its response to antihistamine and bronchodilator drugs. JULIO C. CASARILLO (introduced by EDWIN J. DE BEER), The Wellcome Research Laboratories, Tuckahoe 7, New York. In a paper published in the *Journal of Pharmacology and Experimental Therapeutics*, 90: 104, 1947, a new method is described for magnifying and recording the constrictions and dilatations of the trachea of the guinea pig when exposed to drugs. The technique consists in sectioning the entire trachea into 12 rings of approximately equal thickness and connecting the rings in chain fashion with short loops of thread. The "tracheal chain", as the preparation is called by the authors, is mounted in a tissue bath and connected to a light muscle lever for kymographic recording. "Tracheal chains" made from guinea pigs sensitized to horse serum were found to respond to small concentrations of the antigen with marked contractions which were maintained at the same level for a long period of time. In view of the close anatomical and physiological relation which exists between tracheal and bronchial musculature, these anaphylactic constrictions of the trachea promise to be very useful in the study of drugs which may find application in the relief of certain types of allergic asthma. The anaphylactic spasm, as produced in the guinea pig trachea, dif-

fers from that obtained with histamine mainly in the fact that it cannot be relaxed by either washing nor by the use of antihistamine drugs. Bronchodilator drugs, on the other hand, were found to be very effective in relieving the spasm. These results stir up interesting speculations as to the role that histamine may play in allergic asthma.

**Prolongation of curare action with a peanut oil and beeswax vehicle.** HAROLD F. CHASE and BANI K. BHATTACHARYA (by invitation). *From the Departments of Pharmacology and Surgery, Western Reserve University and The University Hospitals of Cleveland, Cleveland, Ohio.* The efficacy of a beeswax and peanut oil vehicle (Romansky formula) in delaying the absorption of d-tubocurarine has been studied by the rabbit head drop method of assay. Control determination of head drop doses was made in a group of nine rabbits using a preparation containing 0.5 mgm of crystalline d-tubocurarine per cc of distilled water. This preparation was used to titrate the same animals 24 and 48 hours subsequent to the subcutaneous injection of 4.0 mgm per kgm of d-tubocurarine in beeswax and peanut oil, containing 30 mgm per cc. The 34.4 per cent and 10.5 per cent reductions in head drop doses, found, respectively, 24 and 48 hours after the oil injections, were statistically significant and were assumed to indicate the activity of the residual of active curare in the animal. This evidence would suggest that prolonged muscular relaxation of spastic patients following injection of this preparation may be due to true curare action rather than to what has been termed "lissive" action.

**The joint antihistaminic effect and acute toxicity of adrenalin and benadryl HCl.** GRAHAM CHEN, A. C. BRATTON, JR. and CHARLES ENSOR (by invitation). *Research Laboratories, Parke, Davis & Company, Detroit, Michigan.* In histamine induced bronchospasm of guinea pigs, the 50 per cent protective doses of Adrenalin (A), Benadryl HCl (B), and the combination of the two (A + B) were determined by aerosol treatment as follows: A =  $45.31 \pm 5.91 \gamma/L$ , B =  $11.56 \pm 1.41 \gamma/L$  and A + B =  $[21.87 \pm 2.69 \gamma (A) + 5.63 \pm 0.78 \gamma (B)]/L$ . The LD<sub>50</sub>'s of the compounds by intramuscular injection in mice were found to be  $2.93 \pm 0.15$  mgm/kg for (A),  $58.10 \pm 2.21$  mgm/kg for (B) and  $1.20 \pm 0.08$  mgm/kg (A) plus  $22.00 \pm 1.52$  mgm/kg (B) for the combination. Toxic doses of Benadryl HCl produced an increase in blood glucose from 30 to 100 per cent in rabbits. The hyperglycemia was caused by central nervous stimulation and could be prevented by the administration of pentobarbital. Benadryl HCl and Adrenalin given together exerted independently their hyperglycemic effect. The results indicate that the joint antihistaminic and hyperglycemic effect of Adrenalin and Benadryl HCl is additive while the joint lethal toxicity of the two at low dosages of Benadryl HCl is synergistic in nature.

**The electrogram of the papillary muscle.** MAYNARD B. CHILNOWETH and SOLOMON GARB (by invitation). *Dept. of Pharmacology, Cornell Univ. Medical College, New York, N. Y.* An isolated cat papillary muscle was prepared following the method of Cattell and Gold. An additional pair of electrodes recorded the electrogram of the muscle. Studies were made of the effects of fatigue, injury, and rate, and polarity changes. In addition, the effects of such drugs as epinephrine, acetylcholine, chloroform, digitoxin, veratrine, quinidine, caffeine, and procaine on the electrogram and myogram were studied.

**Effect of some derivatives of subtilin on tubercle bacilli and rabbit leukocytes in vitro.** YIN CH'ANG CHIN (introduced by HAMILTON H. ANDERSON). *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, California.* A number of derivatives of subtilin have been prepared by the Western Regional Research Laboratory at Albany, California. The bacteriostatic action of the methylated, ethylated, and glycolated derivatives on *Micrococcus conglomeratus*, *Staphylococcus aureus*, and *Streptococcus faecalis* have been found to be three times that of the original lots of subtilin (personal communication). The original lots and derivatives were submitted to this laboratory for the study of their bacteriostatic action on tubercle bacilli and toxicity to rabbit leukocytes. The bacteriostatic concentrations were determined on human tubercle bacilli, strain H37Rv, in Dubos' fluid medium. This strain was more resistant to subtilin than the strain R1 (reported by the author in Federation Proceedings, 6:317, 1947) which was isolated from an active case of pulmonary tuberculosis by Dr. G. E. Rockwell in 1937 (personal communication) and supplied to this laboratory through the Lilly Research Laboratories at Indianapolis, Indiana. Rabbit leukocytes were obtained by washing them from the peritoneal cavity and the appropriate dilutions of subtilin and its derivatives were made from their solutions in physiologic saline. The results are shown in the accompanying table. It would appear that methylated derivatives were twice as active as the original samples against tubercle bacilli while

| Lot no | Modification of subtilin            | Bacteriostatic concentration against H37Rv (in Dubos medium) | Concentration in which leukocytes showed no apparent change after 60 minutes at 37 C |
|--------|-------------------------------------|--|--|
| 114 Q  | — —                                 | 1 200 000  | 1 1 000  |
| L1318  | — —                                 | 1 100 000  | 1 1 000  |
| L1319  | CH <sub>3</sub> L1318               | 1 200 000  | 1 1 000  |
| L1322  | — —                                 | 1 100 000  | 1 1 000  |
| L1329  | CH <sub>3</sub> L1322               | 1 200 000  | 1 3 000  |
| L1330  | CH <sub>3</sub> L1322               | 1 200 000  | 1 1 000  |
| L1331  | C <sub>2</sub> H <sub>5</sub> L1322 | 1 100 000  | 1 1 000  |
| L1332  | C <sub>2</sub> H <sub>5</sub> L1322 | 1 100 000  | 1 1 000  |

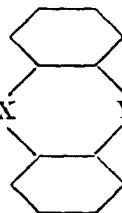
only one of them showed an increased toxicity to leukocytes

**Effects of surface active agents on the activity of pentobarbital and barbital** V V COLL, S H HOPPER (by invitation), and H R HULPILU *Depts of Pharmacology and Public Health, Indiana Univ School of Medicine, Indianapolis, Ind* Mixtures of eleven different surface active agents with a hypnotic dose of sodium pentobarbital were injected intravenously into mice and compared with mice receiving only sodium pentobarbital. In each case the sleeping time was longer with a surface active agent than with sodium pentobarbital alone. The increase in sleeping time varied from 12 to 105 per cent. Seven of these surface active agents were given 5 minutes before sodium pentobarbital and a significant difference from the mixtures was found with only one agent, which gave a greater increase when given before sodium pentobarbital. Another agent (Aresklene 400), which gave the greatest increase by the first method and essentially the same results by the second method, was chosen for study with other animals and with sodium barbital. This agent caused an 82 per cent increase in average sleeping time in rats. Rabbits showed no change in sleeping time but non-fatal doses of sodium pentobarbital proved fatal given with Aresklene 100. In dogs there was a decrease in sleeping time. With sodium barbital as the hypnotic agent there was no difference in sleeping time in the species tested. The time elapsing from injection of sodium barbital to onset of hypnosis was markedly less with Aresklene 400 in mice, rabbits, and dogs while there was no difference in rats.

**Effect of anti-cholinesterases upon procaine toxicity** A C CONWAY (by invitation), F S TRING (by invitation), and JULIUS M COON *Department of Pharmacology, University of Chicago* An enzyme present in blood serum capable of destroying procaine was described in 1943 by Goldberg et al. Kisch demonstrated in vitro that this enzyme was not a cholinesterase but that, like cholinesterase, it could be inhibited by physostigmine and neostigmine. In view of this it was thought of interest to determine what influence these anti-cholinesterase agents might have on the toxicity of procaine. Studies were conducted on mice and rats. The mice were first injected subcutaneously with physostigmine salicylate (0.3 mg/kg) or neostigmine methyl sulfate (0.2 mg/kg). Procaine hydrochloride was administered intravenously via the tail vein about ten minutes later. In the case of physostigmine the LD<sub>50</sub> for procaine rose from 56 mg/kg to 66 mg/kg, thereby indicating a slight protective action of physostigmine upon procaine toxicity. The severity of convulsions due to procaine were also markedly diminished. This same effect was observed in rats and dogs. When neo-

stigmine was tested with procaine an opposite effect on toxicity was noted. The LD<sub>50</sub> for procaine was reduced to approximately 30 mg/kg. Deaths in these instances were practically instantaneous and no convulsant phase occurred. The failure of physostigmine to increase the toxicity of procaine indicates that some factor other than its antiproteinesterase effect operates to influence toxicity. It appears that this mechanism is lacking in the case of neostigmine. This pharmacological difference between physostigmine and neostigmine is being tested in other animal species.

**Inhibition of histamine hypotension by certain heterocyclic substituted ethylamines in the cat** By DONALD L COOK (by invitation), W E HAMBURGER, and MARTIN M WINBURY (by invitation) *From the Pharmacology Laboratories, Research Department, G D Searle & Co, Chicago, Ill* The method described by Wells *et al* (*J Pharmacol* 85:122, 1915) for calculating inhibition of the histamine depressor response in dogs has been used to compare the activities of six compounds with diphenhydramine HCl in cats. These compounds



have the general formula, X

N(CH<sub>3</sub>)<sub>2</sub> Z, where X is S, SO, or SO<sub>2</sub>, Y is  $\lambda$  or CH, and Z is HCl or 8-chlorotheophylline. Preliminary experiments indicated that for each compound histamine antagonism persisted with only slight diminution for at least one hour, permitting the assumption that repeated doses would be cumulative. Three to six animals were, therefore, given doses ranging from 0.025 to 3.2 mg/kg on a geometric scale. In each experiment the hypotension produced by logarithmic doses of histamine di-phosphate from 0.125 to 16.0 gamma/kg was determined before and after each successive dose. From these data, per cent inhibition at each dose level was determined. Curves are obtained when per cent inhibition is plotted against dosage. This curve for DPH in cats is very similar to Wells' curve for DPH in the dog. The data may also be plotted to yield a family of parallel straight lines from which the relative activities of the compounds can be derived. The HCl and 8-chlorotheophylline salts of dimethylaminoethylphenothiazine are the most active compounds in the series.

**A pharmacologic comparison of hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP) with physostigmine and neostigmine** JULIUS M COON and PAUL R SALERNO (by invitation) *University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of*

*Chicago* Hexaethyl tetraphosphite and triethyl pyrophosphite have been reported to be potent cholinesterase inhibitors. A study of the effects of HETP and TEPP on the circulatory system of the cat and dog and on the isolated heart, intestine and uterus of the rabbit showed that these agents are largely similar in action to physostigmine and neostigmine. In anesthetized cats and dogs HETP and TEPP elicited a marked pressor action followed by a cardiac slowing which was prevented or abolished by atropine or procaine but not by vagotomy. The pressor effect was not influenced by nicotization or adrenalectomy but could be abolished by large doses of dibenamine HCl. The isolated heart was relatively insensitive to these drugs, tolerating without effect a 1:10,000 concentration in the perfusing fluid. Larger quantities depressed the activity of the heart. This depression was not affected by atropine. Inserted into the perfusion line, the first dose caused a decrease in coronary flow and subsequent doses caused an increase. The isolated intestine was highly sensitive to these agents. Concentrations of 1:10,000,000 HETP and 1:100,000,000 TEPP, after a latent period, abolished the pendulum movements and established a peristaltic rhythm. This change could be reversed by atropine, procaine, thiarnine and nicotine, but not by repeated washing. The similar effects of physostigmine and neostigmine were easily reversed by washing. The cat and rabbit intestine in situ were also very sensitive to the intravenous injection of HETP and TEPP. The isolated uterus was insensitive to these drugs.

**Total body water by D<sub>2</sub>O tracer study in protein depletion.** F. CO TUI, V. HOLLANDER, A. S. KESTON, J. H. MULHOLLAND. *Department of Surgery, New York University College of Medicine.* The results of a study of the total body water of depleted patients determined by the use of D<sub>2</sub>O as tracer will be presented.

**Protein depletion syndrome and responses to hyperalimentation.** F. CO TUI, J. H. MULHOLLAND, N. KUO. *Department of Surgery, New York University College of Medicine.* The plasma volume and related perimeters were determined in a series of patients with marked weight loss resulting from disease. Significant deviations from normal were found. Some of these patients were then placed on hyperalimentative intake and the evolution of these perimeters toward normal was followed.

**Pharmacological actions of 2-phenylbenzyl-aminomethyl-imidazoline (antistine).** B. CRAVER, W. BARRETT (by invitation), A. CAMERON (by invitation), H. HAYS (by invitation), G. HOLMQUIST (by invitation), A. MACKENZIE (by invitation), J. SMITH (by invitation). *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey.* The following additional data have been secured for Antistine (as the

hydrochloride, sulfate or methane sulfate) which was initially prepared by Miescher, Ulrich and Klarer, and originally studied pharmacologically by Meier and Bucher (*Schweiz med. Wschr.* 76:291, 1946). The intravenous LD<sub>50</sub> for white rats is 39 mg/kg and as with other antihistaminics, convulsions precede death. Rats tolerated 100 mg/kg per os 5 days a week for 30 days with no evidences of toxicity as judged by weight curves and hematological studies. Rats given 10 mg/kg per os 5 days a week for 30 days did not evidence tolerance or accumulative toxicity as judged by subsequent responses to the previously determined LD<sub>50</sub> and LD<sub>50</sub>. Doses of 10 to 50y inhibited isolated, perfused hearts. That of the guinea pig was most sensitive, the hearts of the rabbit and cat least sensitive. The highest concentration that did not inhibit ciliary activity was 1:1000. Five preparations have evidenced the potent antihistaminic properties of this drug. (1) Doses of 100 y/kg markedly inhibited, or briefly eliminated the salivary flow induced by the intra carotid injection of histamine in cats. Lower doses inhibited histamine's sialogogic effect, (2) doses from 10 to 50 mg/kg diminished histamine's hypotensive action from 30 to 40% in dogs, (3) Doses from 1.5 to 3.0 mg/kg eliminated the intestinal spasm induced by intravenously injected histamine (10.20 y/kg) in "Thiry-Vella" dogs, (4) 0.1 y/ml eliminated the stimulating action of 1.0 y/ml of histamine diphosphate upon isolated ileal strips from the guinea pig, (5) Lungs of the guinea pig perfused with solutions containing 1.0 to 10 y/ml became unresponsive to histamine. These data commend the clinical use of the drug. Its therapeutic ratio is high and akin to that of PBZ since Antistine is from 1/2 to 1/16 as active antihistaminically, and about 1/3 to 1/4 as toxic.

**A comparison of the fat storage of the isomers of benzene hexachloride in rats.** BERNARD DAVIDOW, ERNEST C. HAGAN and GEOFFREY WOODARD (introduced by ARNOLD J. LEHMAN). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.* Although in single oral doses the toxicity of the isomers of benzene hexachloride decreases in the order gamma, alpha, delta, and beta, it has been observed in this laboratory that in chronic toxicity experiments the beta isomer is the most toxic. An explanation of this phenomenon was sought by investigating the relative degree of accumulation of each of the isomers in the fat of mature male and female rats after various intervals of feeding on diets containing 100 and 500 ppm of the isomers. The fats were analyzed by a method based upon the conversion of the isomer to 1,2,4 trichlorobenzene and its estimation with an ultraviolet spectrophotometer. Striking differences in the fat storage of the isomers were found on both the 100 ppm and the 500 ppm diets. By far the greatest accumulation was found with the beta

isomer Storage levels of each of the isomers were observed to be higher in females than in males Female rats fed the 100 ppm diets for two weeks exhibited 0.5 and 0.3 mg of the beta isomer per gram of fat, 0.11 and 0.04 of the alpha isomer and no measurable quantities of the gamma and delta isomers Similarly, after 12 weeks on 500 ppm diets, female rats showed the following concentrations in the fat in milligrams per gram fat: beta, 7.5, alpha, 2.4, delta, 0.5, and gamma, 0.4

**Failure of anti-histaminic drugs to antagonise the anemia produced by fat plus choline** JOHN EMERSON DAVIS *University of Arkansas School of Medicine, Little Rock, Ark* Anemia was produced in four dogs by the daily feeding of choline chloride and fat (lard) according to the technique described previously (*Am J Physiol*, 112: 213, 1944; *Science*, 105: 43, 1947) The basic diet consisted of Purina Dog Chow, which we have found effective in maintaining dogs in good health for a number of years Clarkson and Best (*Science*, 105: 622, 1947) reported failure to produce anemia with either choline alone or choline plus fat They used an entirely different diet and therefore did not repeat our experiment Since benadryl and pyribenzamine have been shown to have some anticholinergic potency, and since atropine has been shown to cause remission of our choline-induced anemias, it seemed desirable to determine whether the anti-histaminic drugs would antagonise the anemia caused by fat and choline Therefore, commencing on the third experimental day when the erythrocyte counts had been reduced by one million or more, either benadryl or pyribenzamine was administered orally to the dogs in daily doses of 50 mg The anemias persisted in spite of the administration of the anti-histaminic compounds, showing that the latter are not as effective as atropine in this particular anemia-blocking action

**Some observations on the non-specific choline esterase in the human female** M. EDWARD DAVIS (by invitation), NICHOLAS W. FUGO, and EVELYN AI-FENG YU (by invitation) *From the Department of Obstetrics and Gynecology and the Department of Pharmacology, The University of Chicago and The Chicago Lying-in Hospital* The non-specific choline esterase of sera was studied in pregnant, non-pregnant and gynecologically abnormal women by means of the direct titration method The following observations were made There is wide variation of the values obtained from individual to individual in both the pregnant and non-pregnant group Each individual, however, showed constant values over long periods of time There was no observable increase in non-specific choline esterase titer during gestation Values for the non-pregnant group appear slightly higher than those of the pregnant group although both groups showed the same range of variation Abnormally low

values were obtained in the 2 cases of abruptio placentae studied These values returned to normal postpartum and remained high for a period of 3 months Anemia resulting from hemorrhage or from other causes had no effect on the enzyme titer

**Speed of action of thevetin after intravenous injection in man** CONRADO DAYRIT (by invitation), EDUARDO FARACO (by invitation), NATHANIEL T. KWIT (by invitation), WAITER MODELL, and HARRY GOLD *Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York* Interest was revived by Chen and collaborators in the possible applications of thevetin in cardiac therapy Since no clear evidence exists concerning its proper place in the use of the digitalis series, we re-examined the problem Patients with heart failure and auricular fibrillation were placed at rest in bed After the apex rate and the patient's condition settled down to a constant level, the effect of thevetin was tested either orally or intravenously, frequently by both methods in the same patient The change in the apex rate was the chief guide to the effect of the drug, but it is well known that the decline of the rate follows closely clinical improvement in the congestive failure The results show that thevetin is not satisfactory for oral administration Large single doses of 12 to 30 cat units showed irregular and sometimes negligible therapeutic effects This may be due to either poor absorption and/or rapid elimination The most extraordinary characteristic of thevetin is the speed with which the effects are developed after intravenous injection, the full effect of a dose of from 3 to 6 cat units is developed in about 5 minutes (in typical cases, decline of apex rate from 140 to 100, 120 to 88, 140 to 45) This property may prove of considerable interest in the digitalization of acute heart failure in which a delay of an hour or so may prove decisive The rapid elimination (about 6 hours) adds a factor of safety

**A comparison of side actions and analgesic effects of morphine, amidone, (4-1-diphenyl-6-dimethylamino-heptanone-3) and its isomers in man** JANE E. DENTON (by invitation), OLIVER H. STRAUS (by invitation), WILLIAM E. WADDELL (by invitation) and HENRY K. BECHER *From the Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts* The symptoms and signs, the analgesic effects, and the effects on pulse, respiration and blood pressure produced by a single subcutaneous injection of morphine sulfate (15 mg/150 lb), dl amidone (10 mg/150 lb), l amidone (5 mg/150 lb), iso amidone (10 mg/150 lb) and normal saline (1 cc/150 lb) have been compared in a group of 29 healthy, young ambulatory men, each of whom received each of the five injections at



weekly intervals and was observed for at least five hours following each injection. The doses were chosen for equivalent analgesic effect and for amount necessary to produce sufficient incidence of side actions for statistical analysis. The identity of all drugs was unknown to the subjects and to all except one of the investigators. On the basis of symptom frequencies, times of onset and durations of symptoms, the correlation between morphine and dl amudone, morphine and l amudone, and dl amudone and l amudone varied between  $+0.609 \pm 0.144$  and  $+0.904 \pm 0.033$ , indicating that these three drugs have virtually the same toxicity. Iso amudone, in contrast, had zero correlation with morphine and a correlation with saline of  $+0.918 \pm 0.028$ , indicating that its toxicity is extremely low. Morphine, dl amudone, and l amudone produced statistically significant and equal depression of respiratory rate. Iso amudone produced slight respiratory depression, but much less than the other 3 drugs. Pulse rate was slowed to about the same degree by all drugs. No significant changes in blood pressure were observed. This study clearly shows that morphine sulfate, dl amudone, and l amudone have indistinguishable side effects at dose ratios of 15 to 10 to 5 mg /150 lb of body weight respectively, and that all side effects of dl amudone are carried by the laevo component. The Wolff-Hardy method for pain threshold used in this study did not prove satisfactory in untrained subjects. However, our clinical study of morphine, dl- and l amudone indicates equal analgesic effect of these doses. The analgesic effect of iso amudone is now being assayed clinically.

**Procaine as an anti-allergic agent** ROBERT H. DREISBACH and NAI CHU (Fellow, American Bureau for Medical Aid to China Inc., by invitation) Dept. of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco 15, Calif. Recent reports stressing the use of procaine intravenously in the treatment of serum sickness and urticaria prompted a trial of procaine against experimental sensitizations and histamine toxicity. Guinea pigs were sensitized by the subcutaneous injection of 0.1 cc of horse serum. Three weeks later, 12 of these were injected with 50 mg/kg of procaine subcutaneously. Thirty minutes later, 0.5 cc of horse serum was injected intravenously. Eleven animals died of typical anaphylactic shock. At the same time, 11 sensitized animals were injected intravenously with 0.5 cc of horse serum, and 5 died. Five guinea pigs were injected subcutaneously with 50 mg/kg of procaine, and 30 minutes later injected with 1.0 mg/kg of histamine into the penile vein. All animals died. Five control animals also died from single injections of 1.0 mg/kg of histamine into the penile vein. Eleven rabbits were sensitized (Arthus Phenomenon) by weekly

subcutaneous injection of sodium penicillin G and horse serum. Five of these were injected twice daily with 50 mg/kg of procaine. There were no demonstrable differences in the local reactions resulting from injections of sodium penicillin G (8000 units in 1 cc normal saline solution) or horse serum (1 cc of a 10% dilution in normal saline) between procaine treated and untreated animals. Accordingly, procaine failed to show protective effects against histamine toxicity, and such allergic manifestations as anaphylactic shock and the Arthus phenomenon. Thus the relief obtained with procaine in certain allergic states appears to be subjective, similarly to benadryl and pyribenzamine (Dreisbach J Allergy, in press).

**Relation of meat intoxication in Eck-fistula dogs to hepatic dysfunction** VICTOR A. DRILL, Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. An Eck-fistula was produced in three dogs who were then maintained on a high carbohydrate diet consisting of bread and milk with added iron salts, yeast and fat soluble vitamins. The production of liver damage was followed by the bromsulphalein test. Following a suitable control period the dogs were fed only ground beef heart to produce meat intoxication. As the intoxication developed, no increased impairment of liver function was observed, as measured by the bromsulphalein and alkaline serum phosphatase tests. This was observed even in two dogs that died during the meat intoxication. Two dogs with complete occlusion of the portal vein (occluded in three stages) and two unoperated dogs were similarly fed the beef heart diet but did not develop meat intoxication and also did not show evidence of impaired hepatic function during this period.

**Antidiuretic activity of liver extracts and of urine from patients with hepatic cirrhosis** VICTOR A. DRILL and B. FRAME (by invitation) Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. The concentrated urine from patients with hepatic cirrhosis and ascites showed antidiuretic activity when tested on hydrated rats, thus confirming the results of Rall and associates. Dialysed antidiuretic urine lost activity much more slowly than did dialysed pituitary. The active urines were also without oxytocic activity when tested on the guinea pig uterus. It is not definitely known at present if such antidiuretic urines derive their potency from the posterior pituitary gland. Such a substance may fail to be destroyed by an injured liver. However, the liver must also be considered as a possible source of such antidiuretic activity. When liver extracts were tested it was observed that crude liver extract, purified liver extract (15 USP units/cc), and Intraheptol all had a definite antidiuretic effect. This effect of liver extract was produced by doses

as low as 0.1 cc per 100 grams of body weight, administered intraperitoneally

**Toxicity and mechanism of action of p-nitrophenyl-diethyl-thionophosphate (E605)** KENNETH P. DuBOIS, JOHN DOULL (by invitation), and JUIRUS M. COON *University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago* Lethal doses (20 mg/kg) of E605 (Victor Chemical Works) injected intraperitoneally in cats elicited symptoms of generalized parasympathetic and central stimulation. Onset of symptoms was delayed and death occurred in about 1 hour with primary respiratory paralysis. Autopsy showed an intensely contracted intestinal tract and urinary bladder, and fluid in the respiratory tract. Sublethal doses (5 mg/kg) produced tremors, muscular twitching, and fluid in the respiratory tract, with recovery in about 18 hours. A 90% propylene glycol-10% ethanol solution of the drug (2%) placed in the eye of the cat caused a moderate miosis with delayed onset. Atropine increased the resistance to the drug. In the phenobarbital anesthetized dog 2 mg/kg intravenously gave a 25-fold augmentation of the depressor response to acetylcholine. In tests on rat brain cholinesterase *in vitro* 50% inhibition of activity was obtained by  $1.5 \times 10^{-6}$  M. The approximate LD<sub>50</sub> of E605 given intraperitoneally to rats was 6 mg/kg. After 20 mg/kg 93% inhibition of brain and 78% inhibition of submaxillary cholinesterase activity occurred with an increase of free acetylcholine of the brain from 0.86 micrograms/gm to 3.42 micrograms/gm. To ascertain whether cholinesterase inhibition by E605 is reversible rats were given 5 mg/kg and groups containing at least 5 rats each were sacrificed at various times. The average per cent inhibition of cholinesterase was 94% at 0.5 hours, and in surviving animals was 89% at 1 hour, 46% at 2 hours, and 3% at 4 hours. These experiments demonstrate a reversible cholinergic action by E605.

**The effect of methadon on the cholinesterase of the brain** G. S. EADIL, F. BERNHEIM, and D. B. FITZGERALD (by invitation) *Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina* According to Scott et al. (*J Pharmacol* 91:147, 1947) the effects of methadon on the intestine and heart resemble those of morphine and demerol. The last two drugs inhibit the cholinesterase and the effect of methadon was, therefore, tested. Rat brains were ground in a blender and washed five times in a large volume of acidified water. The preparation from one rat brain was suspended in 25 cc of water and 4.0 cc of this suspension was used in a total volume of 30 cc. The rate of acetylcholine hydrolysis by the enzyme was measured by electrometric titration in the presence and absence of methadon. Methadon definitely inhibits the esterase, and a provisional estimate of the dissociation constant methadon-

esterase indicated that it is of approximately the same order of magnitude as the acetylcholine esterase constant.

**Enzymic hydrolysis of morphine esters** SIDNEY ELLIS *Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina* Atropine, monoacetylmorphine and benzoylcholine are hydrolyzed rapidly by the plasmas of some rabbits, whereas plasmas of other rabbits have little or none of these three activities. It has been shown that atropine esterase is an enzyme distinct from benzoylcholine esterase. It now appears that monoacetylmorphine is hydrolyzed by an enzyme other than those which attack atropine and benzoylcholine. Plasmas of rat (C. I. Wright *J Pharmacol* 75:328, 1942), human, dog, sheep, and guinea pig have negligible activity on monoacetylmorphine. This ester is hydrolyzed by guinea pig liver. Wright found that human and rat livers can attack this compound. The enzyme of rabbit blood is found in the plasma and not in the red cell.

Differences in sensitivities of liver enzymes of rabbits and guinea pigs to heat, acetone drying and inhibition by physostigmine and neostigmine indicate that monoacetylmorphine is attacked by an enzyme distinct from benzoylcholine or tropine esterases. The enzyme which hydrolyzes the alcoholic acetyl group of monoacetylmorphine is able to remove the phenolic acetyl group of the diacetyl compound. However, the enzyme which attacks the latter group is unable to hydrolyze the former. Neither activity is related to acetylsalicylic acid hydrolysis. Specificity for phenolic or alcoholic acetyl groups is not found when phenylacetate and cyclohexylacetate are the substrates. Monoacetylmorphine hydrolysis is not related to methylbutyrate hydrolysis but may be accomplished by the plasma enzyme which attacks tributyrin.

**The therapeutic significance of detectable penicillin levels** N. ERCOLI, M. N. LEWIS (by invitation), B. S. SCHWARTZ (by invitation), M. H. WHITEHEAD (by invitation) *Dept. Pharmacology and Chemotherapy, Warner Inst. Therap. Research, New York City* The duration of therapeutic activity of penicillin is regarded as depending on its presence in antibacterial concentrations in the blood stream. Under the artificial conditions of the laboratory test, the minimum amount which can be detected is 0.015-0.03 units/cc serum; consequently, this concentration is accepted generally as corresponding to that of therapeutic effectiveness. We determined the relation between blood level and therapeutic activity in our study of a new preparation with prolonged duration, which consists of a suspension of crystalline potassium penicillin in oil with vasoconstrictor. First, we determined accurately the duration of the blood level in rats treated with varying doses of this preparation, then we infected the animals at different intervals

when the blood levels were already negative (20-22 hours after treatment). We found that it is possible to protect rats against 100 lethal doses of Pneumococcus Type I, at time periods when the blood no longer contains detectable amounts of penicillin. These experiments indicate that penicillin might be therapeutically effective in serum concentrations below those which can be detected by the bacteriological methods in use, and—more generally—that these *in vitro* methods, though important for comparative purposes, are not suitable for the determination of the absolute value of the therapeutically active blood concentrations. Moreover, we found that with the preparation used the duration of detectable amounts of penicillin in the organs is more prolonged than in the blood. The prolongation of the penicillin tissue levels depends on the vehicle used for injection. For instance, a single treatment with the only suspension with vasoconstrictor gives durations of tissue levels which can be obtained by repeated administration of multiples of the same dose injected in saline. Thus, blood levels and tissue levels are used for injection parallel when different vehicles are used for injection. It might be assumed that a more prolonged duration of penicillin in the organs will produce relatively longer periods of therapeutic effectiveness.

**Comparison of depth of local anesthesia obtained with 2% and 4% procaine solutions** FRANK G. LEBRETT (introduced by NORMAN DAVID) Department of Pharmacology, University of Oregon Medical School, Portland, Oregon. Clinical experience indicates that for certain dental procedures 4% procaine solutions have advantages over the recommended 2% solutions for infiltration anesthesia. Such advantages have been difficult to measure by previous laboratory techniques which are concerned primarily with onset and duration of local anesthesia rather than with intensity or depth. We have compared local anesthetic potency in two homologous teeth of the upper arch of dogs lightly anesthetized with sodium pentobarbital. Shallow occlusal cavities were prepared in each tooth to such a depth that equal minimal responses were noted when their sensitivity was tested by a weak faradic current of an inductorium. Simultaneous stimulation of each cavity revealed that after the first 10 minutes following nerve block with 4% procaine, responses were consistently absent or reduced to a stimulus which would produce a definite pain response in the contralateral tooth blocked with 2% procaine. This objective confirmation of the clinical advantages of 4% procaine solutions suggests the need for investigating the relative dangers of 2% and 4% procaine when so used. Such a study is now in progress.

**Studies on the toxicity and metabolism of d-tubocurarine** G. M. EVERETT (introduced by R.

K. RICHARDS), Dept. of Pharmacology, Abbott Research Laboratories, North Chicago, Illinois. There is considerable difference in the paralytic dose of d-tubocurarine for various species. The paralytic dose in mg/kg for rabbits is 0.15, dogs 0.2, cats 0.2, mice 0.125, rats 0.075, guinea pigs 0.035. Early paralysis of the respiratory muscles and the development of myoclonic jerks and convulsions are observed most often in mice, rats and guinea pigs. Artificial respiration with added oxygen aborts these anoxic effects. In anesthetized and unanesthetized cats and dogs just paralytic doses cause a transitory fall in blood pressure of 10 to 25 mm. Higher doses caused a marked vasodilation of 10 to 60 mg with little recovery of the blood pressure. Vasopressor drugs are effective in restoring blood pressure to normal levels. No direct cardiotoxic effects were observed with doses up to 50 times the paralytic dose. Both the ECG and EEG remained essentially normal. There were no differences in the intravenous paralytic dose or duration of action observed between control, nephrectomized, hepatectomized rats (80%), or nephrectomized hepatectomized rats. Studies in similarly operated rabbits showed no increase in sensitivity or duration of action of d-tubocurarine from normal controls. Upon giving a second dose after two hours to normal and operated animals no sign of residual curare was observed. These experiments indicate that the kidneys and liver are not essential for the metabolism of d-tubocurarine.

**Some factors influencing the activity of cardiac glycosides in the rat** A. FARAH and E. SZYMUSZKOWICZ (introduced by Dr. JAMES M. DILLE) Departments of Pharmacology, University of Washington Medical School, Seattle, Washington and the American University of Beirut, Beirut, Lebanon. The toxic dose of g-Strophanthin is over a thousand times greater in the rat than in the cat. This is mainly due to the high elimination rate and the high resistance of the rat heart to the cardiac glycosides. The following factors concerned with the elimination and lethal dose of g-Strophanthin were studied: Rate of administration. A reduction in rate increases the lethal dose and experimental time. Anesthesia. Amytal and Urethane have been compared. With Urethane anesthesia the lethal dose is about seventy per cent lower than with amytal anesthesia. Functional hepatectomy. Markedly reduces the lethal dose and elimination of g-Strophanthin. Liver damage. Carbon tetrachloride in doses sufficient to produce liver damage reduces the lethal dose of g-Strophanthin. Concentration of g-Strophanthin. If the rate of administration of g-Strophanthin is maintained at a constant value, the lethal dose is reduced by about 50% if the concentration of the glycoside infused is reduced from 5 to 2.5 mg per cc.

**Absorption and potency of small divided doses of dicumarol in man** DAVID W. FASSETT and E.

STERLING NICHOL (by invitation) *Cardiology Service, James M. Jackson Memorial Hospital, Miami, Fla.* During a recent study by Nichol and Fassett (*Jour. Southern Med. Ass'n* 40: 631, 1947) on the use of dicumarol over long periods of time to prevent recurrences of coronary thrombosis, considerable difficulty was encountered when attempts were made to estimate daily dosage requirements for periods of a week or two in advance. The available dosage forms of 50 or 100 mgm. capsules, while satisfactory for short term use of a few weeks, were entirely too cumbersome to allow a precise adjustment of daily dosage. In ten patients studied over periods varying from 8 to 11 months, the daily dose of dicumarol necessary to cause a reduction of plasma prothrombin content to about 20% of normal varied from 25 to 150 mgm. per day, and bore little relation to weight, diet, or other medications. By using 25 mgm. capsules given in divided doses throughout the day, it was found that the total daily requirement was the same or less than when the drug was given in single doses, and that in most cases there was less fluctuation in prothrombin level of the blood. It seems reasonable to assume that drugs such as dicumarol and propylthiouracil which presumably interfere with normal chemical processes, should be given in such a manner as to be present at the site of action in fairly constant amounts.

**Effect of various compounds containing iodine and other halogens on thiouracil goitre.** J. K. W. FERGUSON and E. A. SELLERS. *Department of Pharmacology, University of Toronto.* Goitres were produced in rats weighing about 150 gms. by giving thiouracil (0.1%) in the drinking water. The compounds studied were either added to the drinking water or injected subcutaneously. After two weeks of treatment the rats were killed and the thyroid glands weighed. Thyroxine by injection prevented completely the enlargement of the thyroid gland by thiouracil. Sodium iodide in daily doses ranging from 0.03 mg. to 0.1 gm. decreased the enlargement by 20-30%. Iodoacetic acid (0.04-5 mg.) produced a similar effect. Diiodotyrosine by injection had no more activity than could be attributed to its content of iodine. Sodium chloride up to 0.1 gm./day had no effect, while sodium fluoride 0.6-18 mg./day increased appreciably the enlargement of the gland. An increase in size also occurred with sodium bromide (0.004-0.1 gm./day), although this was less marked than with fluoride. In spite of the effect of iodide on gland size, the decline in metabolic rates of animals receiving iodide with thiouracil did not differ from those of animals receiving thiouracil only. It is postulated that the action of iodides is antithyrotropic rather than by direct interference with the action of thiouracil. Studies are continuing on the effect of prolonged administration of iodides.

**Effect of scilliroside on the respiration of cat heart muscle.** MURRAY LINKISER (by invitation) and OSCAR BODANSKY. *Dept. of Pharmacology, Cornell University Medical College, New York City.* Scilliroside increased the rate of respiration of slices of cat heart muscle in the presence of glucose and in a reaction medium containing 18 mM phosphate and 0.8 mM calcium per liter. A concentration of  $1.6$  to  $2.5 \times 10^{-3}$  M caused a slow but continuing increase, two hours after the introduction of scilliroside the rate had increased by about 20%. A concentration of  $10^{-2}$  M scilliroside led to an increase of the same magnitude within the first hour, higher concentrations ( $10^{-2}$  to  $5 \times 10^{-2}$  M) led to maximal increases of about 40% within the first 30 minutes. In all these instances, the increases were sustained during the period of observation. In the absence of calcium, the introduction of scilliroside ( $1.6 \times 10^{-3}$  M) caused a decrease of about 20% in the rate of respiration. In reaction media containing 0.8 or 1.6 mM calcium per liter but only 1.8 mM phosphate per liter, the rate of respiration was less than in the usual medium, the introduction of scilliroside caused marked increases of about 80 to 100%. In the absence of calcium and with low concentrations of phosphate (1.8 mM per liter) the rate of respiration was essentially normal, the introduction of scilliroside caused a decrease of about 10%.

**Comparative tolerance of dogs, cats and rabbits to nicotine.** J. K. FINNEGAN, P. S. LARSON and H. B. HALL. *Department of Pharmacology, Medical College of Virginia, Richmond.* As part of a program concerned with determining the fate of nicotine in the body, we have compared the detoxification capacity for it on several species. Two series of determinations were made on barbitized animals: (1) LD<sub>50</sub> values as determined by instantaneous intravenous injection of the total dose and (2) the dose required to produce respiratory paralysis when given over an 8 hour period by continuous intravenous infusion. LD<sub>50</sub> results were as follows: cats, 2 mg. per kg.; dogs, 5 mg. per kg.; rabbits, 9.5 mg. per kg. The results by the second series of determinations were: dogs, ca. 15 mg. per kg.; cats, ca. 22 mg. per kg.; rabbits, ca. 40 mg. per kg. Subtracting the values obtained in (1) from those obtained in (2), it is found that the capacity of these species to detoxify and excrete nicotine, in terms of mg. per kg. per 8 hours, is approximately as follows: dogs, 10; cats, 20; and rabbits, 30.

**The chronic toxicity of thiourea.** O. GARTH FITZHUGH, ARTHUR A. NELSON and OMA L. HOLLAND (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.* Following the demonstration that orange decay was controlled by thiourea and that the juice of treated oranges contained thiourea (J. F. L. Childs and

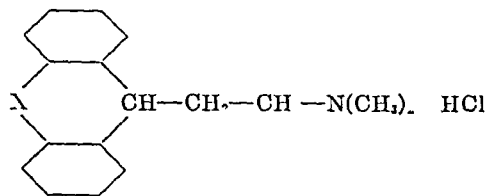
E. A. Siegler, *Ind and Eng Chem*, 38 S2, 1916), a 2-year chronic toxicity study was started. Albino rats, 21 days old, 18 to the group, were fed thiourea at levels of 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.001% in a stock diet. All rats receiving 1, 0.5 and 0.25% thiourea died within the first year. Significant retardation in growth occurred at dosages of 0.025% or more. Dosages of 0.25% or more produced marked enlargement and, microscopically, marked hyperplasia of the thyroid. The 0.05 and 0.1% thiourea produced significant enlargement and hyperplasia of the thyroid. At all levels below 0.25% the production of liver tumors in the later months of the experiment was a more outstanding effect than were the thyroid changes. No tumorigenic effect of thiourea was seen at 0.25% or above, presumably because of the early death of all animals in these groups.

**Statistical problems involved in the use of radioactive tracers in pharmacology.** REX G. FLUHARTY (introduced by LLOYD C. MILLER). *Radioactivity Center, Massachusetts Institute of Technology*. The paper will first present a short discussion of the probability of radioactive disintegration and its effects upon the half life of the isotope, the statistics of the measurement, and the measurement sensitivity. An analysis will be made of some typical Geiger counter data. Limitations imposed by statistics will be pointed out, and criteria will be given for good operating conditions. Finally a summary will be given including the statistics of experimental factors such as sampling variations and limitations imposed by the techniques used.

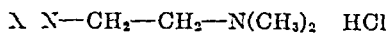
**Effects of certain "cardiac stimulant" drugs on coronary circulation and cardiac oxygen metabolism.** ELWOOD L. FOLTZ, SAU KI WONG and JAMES E. ECKENHOFF (introduced by CARL F. SCHMIDT). *Department of Pharmacology, School of Medicine, University of Pennsylvania*. The effects of four "cardiac stimulant" drugs on the coronary circulation and the oxygen metabolism of the heart were studied in 16 experiments by a previously reported technique in intact dogs lightly anesthetized with pentobarbital sodium. Blood  $O_2$  and  $CO_2$  content was determined from samples obtained by catheterization of the coronary sinus, the pulmonary conus, and by direct puncture of the femoral artery. Respiratory studies of  $O_2$  and  $CO_2$  were accomplished on expired air collected in a Tissot spirometer. Cardiac output was estimated by the direct Fick method. Coronary flow was estimated by the  $N_2O$  technique as adapted to the coronary circulation (*Am J Med Sci*, December 1947). After obtaining control values for cardiac output and coronary flow, one of the following drugs was injected intravenously and studied: theophylline with ethylene diamine, papaverine hydrochloride, nikethamide, and khellin. Pre-

liminary studies have shown that theophylline with ethylene diamine increased coronary flow in 2 of 3 experiments, but cardiac work and  $O_2$  consumption was increased in all three. Papaverine decreased  $O_2$  consumption in 4 experiments while coronary flow was increased only in the 2 experiments where a large dose (3 mgm per K) was injected, cardiac work was uniformly decreased. The response to nikethamide was variable. In 2 experiments with khellin, no significant increase in coronary flow was observed.

The effectiveness of certain substituted ethylamines against histamine spray-induced fatality in the guinea pig. HOWARD B. FREESE (by invitation), W. E. HAMBOURGER, and PATRICIA M. MICHIELS (by invitation). *From the Pharmacology Laboratories, Research Department, G. D. Searle & Co., Chicago, Ill.* Three series of compounds have been studied for antihistamine activity on guinea pigs using the histamine spray technique described by Loew, Kaiser, and Moore (*J Pharm and Exp Therap* 83 120, 1945). Animals were treated by intraperitoneal injection fifteen minutes before exposure to the histamine mist. Groups of untreated controls were run each day. The compounds studied have the following general formulae:



Series I



Series II

In both series, X may be oxygen, sulfur, methylene, C-C linkage (i.e., diphenylene), or by no linkage (diphenyl). The two series, therefore, differ only in the methylene or nitrogen bond between the nucleus and the  $\beta$  dimethylaminoethyl side chain. Series III consists of the 8-chlorotheophylline salts of the more active members of Series I and II. The MED is taken as the lowest dose which reduces the per cent mortality significantly when compared with the control mortality for the day. The MED of diphenhydramine HCl is arbitrarily assigned an activity index of 1, and on that basis indices are calculated for each of the compounds. The most active compounds are those in series I and II in which X is either sulfur or methylene, and their respective 8-chlorotheophylline salts in series III. The acridan compound (Series II, X =  $\text{CH}_2$ ) is outstanding with an activity index of 16.

**The effect of tetraethylammonium on the pressor response to anoxia and asphyxia** WALTER FREYBURGER (by invitation), LUIS R. CARO (by invitation), and GORDON K. MOL From the Department of Pharmacology, the University of Michigan, Ann Arbor The pressor response to anoxia and asphyxia is thought to be due to central and reflex excitation of vasopressor mechanisms, including the secretion of epinephrine Since the humoral mechanism of excitation of the adrenal medulla is cholinergic and analogous to the ganglionic synapse, and since TEA blocks the pressor action of high doses of acetylcholine, it was expected that this drug would prevent the hypertensive response by interrupting transmission between nerve ending and adrenal medullary cell In the cat and rabbit, the pressor response was blocked by TEA, but in the dog the drug did not prevent, and often even potentiated, the hypertensive reaction That some humoral pressor agent was indeed liberated in the dog in the presence of TEA was demonstrated, for vasoconstriction occurred in the denervated leg during exposure to asphyxia That this humoral agent is epinephrine is likely, for Dibenamine, which converts the pressor action of epinephrine to a depressor action, also converts the pressor response to asphyxia to a depressor response, even in doses which fail to block completely the effects of sympathetic vasoconstrictor nerve stimulation It is probable, then, that TEA fails to prevent the liberation of epinephrine in asphyxia In the light of recent experiments of Bulbring, Burn, and de Elho on the effects of local anoxia on the adrenal medulla, it would conclude that although TEA probably prevents neurogenic stimulation of the gland, anoxia and asphyxia may produce in the dog a direct excitation which cannot be blocked by TEA

**Studies on ventricular fibrillation produced by epinephrine during hydrocarbon inhalation** SOLOMON GARB (by invitation) and MAYNARD B. CHENOWETH Dept of Pharmacology, Cornell Univ Medical College, New York, N. Y. Experiments performed on 20 cats show that a sudden rise in systemic arterial pressure is not necessary for the production of ventricular fibrillation by epinephrine during hydrocarbon inhalation Dibenamine protected 8 out of 10 cats from ventricular fibrillation in doses (3 mgm/kgm) which did not reverse the pressor effect of epinephrine Nor-epinephrine induced ventricular fibrillation during hydrocarbon inhalation in 5 out of 5 cats, while n-isopropyl epinephrine failed to induce it in 9 out of 9 cats, although in 4, the systemic arterial pressure was raised by aortic constriction In 5 out of 5 isolated perfused cat hearts, ventricular fibrillation was produced by benzene and epinephrine Experiments on the

threshold of irritability of papillary muscles show that benzene and chloroform raise the threshold very markedly, while ethyl ether, ethyl alcohol and acetone raise it very slightly Epinephrine produces spontaneous rhythms in those papillaries which have a small pacemaker nodule at the base Analysis of 11 electrocardiograms taken at the onset of ventricular fibrillation reveals that there is no definite acceleration before the onset of fibrillation A characteristic pattern was noted in each record, consisting of a small QRS followed by a large T wave, with ventricular fibrillation beginning at the peak of the T wave These findings suggest that ventricular fibrillation is initiated when in area of myocardium containing a slightly higher concentration of hydrocarbon than the remainder of the heart fails to respond to a stimulus (QRS) but does respond to the following T wave

**Synergism in relation to toxicity of certain antiasthmatic drugs** R. A. GARDNER (by invitation), P. L. EWING, G. A. EMERSON and A. E. HANSEN Death of an infant following the accidental consumption of what was believed to be sublethal amounts of the components of a proprietary preparation prompted the present study Lethal levels of the component drugs, ephedrine, theophylline and phenobarbital, were quantified by intraperitoneal injections into a total of 507 healthy young adult white mice of a stock strain The LD<sub>50</sub> as estimated by the method of Miller and Tainter was found to be for ephedrine sulfate 307 ± 9.6 mg/kg, aminophylline 256 ± 3.5 mg/kg, and phenobarbital Na 192 ± 11.1 mg/kg Inasmuch as it was not believed that the patient suffered from phenobarbital intoxication and since a few preliminary tests indicated a marked potentiation of ephedrine by aminophylline, combinations of varying fractions of the LD<sub>50</sub> of each of these latter two drugs were tested One fourth of the LD<sub>50</sub> of ephedrine plus 140 mg/kg of aminophylline gave a mortality rate of 80%, indicating more than a four-fold increase in the toxicity of ephedrine by the concomitant action of sublethal doses of aminophylline, or a doubling of the toxicity of aminophylline by a sublethal dose of ephedrine Phenobarbital decreases toxicity of both agents, alone or in combination

**Inhibitory effects of naphthoquinones and related compounds on glycolysis** CHALMERS L. GEMMILL Department of Pharmacology, University of Virginia, Medical School, Charlottesville, Virginia A study was made of the inhibitory action of some naphthoquinones and related compounds on the conversion of glycogen to lactic acid by extracts of frogs' muscles The rate of glycolysis was determined by the liberation of carbon dioxide from a bicarbonate buffered solution in a Warburg vessel It was found that the following compounds

partially or completely inhibited glycolysis in concentrations of 0.003 M or less Sodium 1,2-naphthoquinone-1-sulfonate, 2-methyl-3-bromo-1,4-naphthoquinone, 2-chloro-3-(n)thiobutyl-1,4-naphthoquinone, hydroquinone, 2-methyl-3-thioethyl-1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone. The following compounds did not inhibit glycolysis in amounts of 0.003 M or less: Lawsone, Lypachol, Lomatol, 1,4-naphthoquinone-2-thiopropionic acid, 2-methyl-3-isothiobutyl-1,4-naphthoquinone, 2,3-dimethyl hydroquinone, 2,3,5-trimethyl hydroquinone, 2-methyl hydroquinone. It was necessary to dissolve some of these substances in small amounts of alcohol before adding to the enzyme mixture. Control experiments demonstrated that this amount of alcohol had no effect on the rate of glycolysis. The addition of cysteine reversed partially the inhibitory action of sodium 1,2-naphthoquinone-1-sulfonate.

**Inhibitory effect of stilbamidine, guanidine and arginine on glycolysis.** CHALMERS L. GEMMILL, *Department of Pharmacology, University of Virginia, Medical School, Charlottesville, Virginia*. Read by title. It was found that stilbamidine diisothionate (Merck), guanidine hydrochloride (Eastman) and arginine monohydrochloride (Bios Laboratories) inhibited the breakdown of glycogen to lactic acid in extracts of frogs' muscle. The concentrations used were 0.003 M or less. Guanidine hydrochloride inhibited this reaction only when it was added before glycolysis was started. When it was added twenty minutes after the glycogen was placed in the reaction vessel, no inhibitory action was found. Other amidines and guanidines are now under investigation.

**Malaria chemotherapy.** 1. The response of trophozoite-induced infections with *Plasmodium cynomolgi* to various antimalarial drugs. CLARA S. GENTHER (by invitation), WANDA SQUIRES (by invitation), ROCHELLE FRADKIN (by invitation) and L. H. SCHMIDT, *Christ Hospital Institute of Medical Research, Cincinnati, Ohio*. This report is part of a study designed to determine whether infections with *Plasmodium cynomolgi* in the rhesus monkey can be utilized in the systematic search for chemotherapeutic agents which will cure human infections with *P. vivax*. This paper deals with the response of trophozoite-induced infections with *P. cynomolgi* to such drugs as quinine, quinacrine, chloroquine, oxychloroquine, and chlorguanide (Paludrine). More than 200 monkeys were infected with measured numbers of trophozoites. Treatment with one of the above drugs was initiated at various stages of the primary attack or during the first or second relapse in instances where the initial treatment was sub-

curative. The standard treatment period was 7 days. All drugs except quinine were administered once daily, quinine was administered both in single daily doses and at 6 hour intervals. Infections were considered cured when the blood remained free of parasites (by thick film examination) for 12 consecutive weeks after treatment, despite protein shock and splenectomy. Under the above conditions the daily doses (mgm per kgm) required for systematic cures were: quinine, 80; quinacrine, 10; oxychloroquine, 5; chloroquine, 2.5; chlorguanide, 0.6. Using quinine as the reference standard, the relative activities of the other drugs were: quinacrine, 8; oxychloroquine, 16; chloroquine, 32; chlorguanide, 128. These ratios compare with approximate values of 2, 4, 6, and 60 for the respective drugs in *P. vivax* infections. It may be concluded, therefore, that trophozoite-induced infections with *P. cynomolgi* and *P. vivax* are relatively similar in their response to the above drugs.

The efficacy of BAL (2,3-dimercaptopropanol) in the treatment of experimental lead poisoning in rabbits by FREDERICK G. GERMUTH, JR. (by invitation) and HARRY EAGLE, *From the Laboratory of Experimental Therapeutics of the U. S. Public Health Service and the Johns Hopkins School of Hygiene and Public Health, Baltimore 5, Maryland*. Acute and subacute lead poisoning were produced in rabbits by the administration of lead acetate. Five consecutive daily subcutaneous injections of lead acetate at a dosage of 240 mg of compound per kg per injection regularly caused the death of rabbits within 3 to 40 days. The intravenous injection of the same salt at a dosage of 12 mg/kg per injection, repeated every 3 hours for 4 doses, produced an acute poisoning from which the animals died in 1 to 18 days. The intramuscular administration of BAL in peanut oil and benzyl benzoate, at individual dosages of 5 to 20 mg/kg, repeated every 4 hours, failed to protect these animals. In one group of animals with subacute (subcutaneous) lead poisoning, rabbits treated with BAL died significantly faster than did the corresponding untreated controls. The administration of BAL, however, caused a marked increase in the urinary excretion of lead. For 2 hours after a single injection of BAL at 20 mg/kg, the urinary excretion of lead increased 11 to 40 fold in animals with a subcutaneous depot, and 3- to 7 fold in animals injected intravenously. However, the magnitude of the excretion response diminished with each additional injection of BAL. Treatment with BAL had no demonstrable effect on the histopathological lesions produced by lead. It is believed that the failure of BAL to protect lead poisoned animals may be in part due to the fact that it mobilizes

only a small fraction of the total body store of lead, and in part to the fact that the lead BAL complex proved almost as toxic as the lead salt itself on intravenous injection

**Toxicity and cardiotonic activity of antibiotic lactones and synthetic analogs on the isolated frog heart** N J GIARMAN (introduced by E L MCCAWLEY) *From the Laboratories of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut* A series of 30 lactones and synthetic analogs, some of which have been shown to possess striking antibacterial, antiprotozoal, and antiviral properties, have been studied to ascertain their cardiac toxicity and cardiotonic activity, known to be inherent in the lactone configuration. A special cannula introduced by Kriyer as a modification of the Straub technique has been used in the entire investigation. The toxicity experiments revealed the important generalization that the unsaturated lactones were by far more cardiotoxic than either the saturated lactones or the furans tested. Three of the parent-structured unsaturated lactones were chosen for a more intensive study of the time for systolic standstill concentration relationship with ouabain and digitoxin as points of reference. Thus if the minimum millimolar toxic concentration of ouabain is arbitrarily designated as 1, that of 3-pentene-1,4 olide is 60, that of 2-pentene-1,4 olide is 600, that of the dilactone of pulvic acid is 1, while that of digitoxin is 3. Several significant findings were disclosed by the experiments designed to show a cardiotonic effect on the frog heart made hypodynamic with low-calcium Ringers. All of the saturated lactones were devoid of activity except two 2-keto-3-dimethylaminomethyl-butyrolactone and 2-keto-3-(3,3-dimethylacryl)-butyrolactone, which bore the respective ratios of 1:1486 and 1:2570 in relation to the minimum millimolar positive inotropic concentration of ouabain. The most effective unsaturated lactones were the dilactone of pulvic acid (1:21), 3-phenyl-2-butene-1,4 olide (1:292), 1-ascorbic acid (1:335), and 3-pentene-1,4 olide (1:600). The structurally related gamma-pyrones, kojic acid and meconic acid, yielded the positive inotropic ratios of 1:3400 and 1:327 respectively. The other cardiac glycosides used as reference agents were digitoxin and uzarin which showed the positive inotropic ratios of 1:2.4 and 1:7.0 respectively.

**Distribution and excretion of benadryl ( $\beta$ -dimethylaminoethyl benzhydryl ether)** A J GLAZKE, W A DILL (by invitation), and D A MCGINTY *Research Laboratories, Parke, Davis and Co., Detroit, Michigan* Benadryl concentrations were determined in the tissues of the rat and guinea pig at various time intervals after oral and parenteral administration. The drug

was extracted from alkaline tissue homogenates with heptane, and determined chemically by a modification of the methyl orange procedure described by Brodie, Udenfriend and Dill (*J Biol Chem*, 168:335, 1917). Maximum concentrations of Benadryl were found 1 to 2 hours after subcutaneous administration. Guinea pig tissue showed about 25 times the concentration of Benadryl found in rat tissue. In both species, lung tissue showed by far the greatest concentration of Benadryl, followed in order of decreasing concentration by spleen, kidney, liver, brain, skin, muscle and blood.

Excretion of the drug and its degradation products was also investigated in rats, using radioactive Benadryl with carbon-14 in the alpha position of the benzhydryl group. About 50 percent of the administered radioactivity was recovered in the first 24 hour urine specimens, principally in the form of a non-basic degradation product. The degradation process was also investigated *in vitro* with tissue slice preparations. A small amount of unaltered Benadryl was found to be excreted in human urine, using the Craig counter-current distribution technique for extraction and identification of the compound.

**The binding of barbiturates by human and bovine serum albumin** By LEO R GOLDBAU (by invitation) and PAUL K SMITH *From the Department of Pharmacology, The George Washington University School of Medicine, Washington, D C* Using an ultratiltration technique described by Lavielles (*J Biol Chem*, 120:267-75, 1937) some of the factors affecting the binding of several barbiturates and thiobarbiturates by albumin have been investigated. The extent of binding varies with the pH, passing through a maximum at about pH 7.6-8.0 for all the drugs investigated. Increasing the albumin concentration from 0.5 percent to 5.0 percent increased the amount of pentothal bound from 30 percent to 85 percent. With increasing pentothal concentrations, the percent bound decreased from 90 percent at 50 mgm per liter to 50 percent at 900 mgm per liter. Thiobarbiturates are bound to a greater extent than their oxygen analogues (barbital 5 percent, thiobarbital 39 percent, pentobarbital 40 percent, pentothal 65 percent). At a constant pH of 7.3 and constant drug and phosphate buffer concentration, the percent bound usually increased with the length of the substituent groups (barbital 5 percent, ipral 13 percent, neonal 28 percent, pentobarbital and amytal 37 percent and orlatal 65 percent). Those with a cyclic side chain were intermediate (phenobarbital 20 percent, phanodorn 25 percent, cyclopal 31 percent, evipal 29 percent). Allyl groups instead of ethyl groups increased binding slightly (dial 13 percent, alurate 20 percent, seconal 44 percent). Work is in progress.



to determine if the extent of protein binding is associated with the relative concentrations in the plasma and tissues

**Studies on diffusion respiration VII Electrical cortical activity in dogs** ELI S. GOLDENSOHN (by invitation), EWALD W. BUSSE (by invitation), JOSEPH N. SPENCER (by invitation), WILLIAM B. DRAPER and RICHARD W. WHITEHEAD *From the Department of Physiology and Pharmacology and the Department of Psychiatry, University of Colorado Medical Center* Respiratory arrest was induced and maintained for forty-five minutes in ten dogs according to the technique of Draper and Whitehead (1944). E. C. G. records were examined before, during and for two months after the procedure. Pre diffusion control and post diffusion E. C. G.'s were taken under light thiopental sodium anesthesia. The pH of the superior vena caval blood averaged 7.32 at the onset of diffusion respiration and 6.69 after forty-five minutes of apnea. In the same period the blood sugar rose from an average of 91.9 mg per cent to 150.3 mg per cent. In the first few minutes of diffusion respiration the cortical waves decreased in amplitude but the frequency was not altered. In the next few minutes the cortical rhythm decreased in both amplitude and rate. Between five and fifteen minutes, intermittent periods of complete absence of electrical activity appeared. These periods lengthened progressively until at about fifteen minutes electrical activity could not be recorded even at the highest amplification. At this time the blood pH averaged 6.95. Complete absence of electrical activity persisted for the remainder of the period of apnea. On the resumption of respiration electrical cortical activity was seen in a few moments. This activity was slow and irregular for the first several hours. The E. C. G.'s appeared similar to the controls within two weeks after diffusion. The animals were sacrificed two months after the experiment. Pathological studies of the brain and other organs are being made.

**Inhibitors of purified human plasma cholinesterase** AVRAM GOLDSTEIN (introduced by OTTO KRAYER) *Department of Pharmacology, Harvard Medical School* An investigation into the inhibition of purified human plasma cholinesterase (fraction IV 6.3 of the system of Cohn et al., J. Am. Chem. Soc., 68: 459, 1946) by a variety of compounds belonging to widely differing chemical classes. A manometric method was used. It has been shown (Goldstein, A. J. Gen. Physiol., 27: 529, 1944) that one molecule of physostigmine combines reversibly with one molecule of dog serum cholinesterase and that the inhibitor competes with acetylcholine for the active group of the enzyme. The compounds studied here can be similarly classified as reversible or irreversible.

Reversible inhibitors can be further described as competitive or non competitive with respect to acetylcholine. Mercuric and cupric ions inhibit irreversibly. Methylene blue, acriflavin, morphine, atropine, strychnine, amphetamine, phenobarbital, sulfamidamide, procaine, choline, acetyl- $\beta$  methylcholine inhibit reversibly but non competitively. The 1:1 relationship of molecules inhibitor to enzyme active centers applies here. Since the interaction is reversible yet unaffected by the presence of substrate (acetylcholine), we assume the enzyme is inhibited by interaction at some point other than the usual substrate combining center. Physostigmine, prostigmine and carbaminoylcholine inhibit reversibly and competitively—i.e., are displaced from combination by acetylcholine. We take this to indicate interaction at the substrate combining center. These fundamental differences in mechanism of inhibition can not be correlated with the molar potencies of the inhibitors. Consequently molar potency (or dissociation constant) and molar combination ratio do not adequately describe an inhibitor. Further classification as reversible or irreversible and competitive or non competitive facilitates a more rational approach to the problem of relationship between specific structural configuration and inhibition capability.

**Chemotherapeutic studies on a series of dithiocarbamates and their bismuth derivatives** ANDRES GOTH and FABIAN J. ROBINSON (by invitation) *Dept. of Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas* One of us has reported that bismuth forms a highly active complex with "aspergillic acid". Further studies have shown that certain dithiocarbamates behave in a manner similar to "aspergillic acid" in being potentiated by bismuth. The dithiocarbamates studied were (in decreasing order of activity against *Staph. aureus*) Diethyl-, dimethyl-, morpholyl-, and isoamylethyl. Sodium diethyl dithiocarbamate inhibited the growth of *Staph. aureus* in a dilution of 1:500,000. Some of the bismuth derivatives were much more active than the parent compounds. Bismuth diethyl dithiocarbamate inhibited the growth of *Staph. aureus* in a dilution of 1:10,000,000. Much of this activity was retained in 50% human plasma. The compound has a wide antibacterial spectrum, inhibiting *M. tuberculosis* and *E. coli* as well as Gram positive organisms. Inhibitory blood levels of 20–40 Staph. Units were obtained in two dogs which received 20 mg per kg of bismuth diethyl dithiocarbamate intravenously. Demonstrable blood levels persisted for 30 minutes. When bismuth thioglycollate and sodium diethyl dithiocarbamate were given by separate intramuscular injections to dogs inhibitory blood levels of 10–20 Staph. Units could be maintained for at least 2 hours. The

MLD of sodium diethyl dithiocarbamate for mice was found to be greater than 500 mg per kg intraperitoneally. Animal protection experiments are under consideration and will be reported.

**Factors influencing the hypertensive action of desoxycorticosterone** D M GREEN and M GLOVER (by invitation) *School of Medicine, University of Washington*. Single 20 mg pellets of desoxycorticosterone were inserted subcutaneously in 30 young rats. Twenty-four additional animals received 10 pellets each. Equal numbers of males and females were used. Half the animals in each group were adrenalectomized. Blood pressure, weight and fluid intake were observed for 3 months. During the first 3 weeks all animals received isotonic saline to drink to minimize the post-operative mortality in the adrenalectomized animals. Thereafter, water was given to alternate animals. The immediate consequence of implantation was an abrupt rise in fluid intake, maximal within 10 days, followed secondarily by slow reversion toward control levels. Blood pressure showed no pronounced initial reaction but rose slowly during the three month period. In general, pressure elevation followed the initial rise in intake, and appeared inversely related to the secondary fall. At the 10 pellet dose level, major differences in degree of hypertension attributable to sex, salt intake or adrenalectomy were not demonstrated. At the 1 pellet level, maximum pressure in male animals definitely exceeded that in females, the majority of which failed to develop significant hypertension.

**The effects of 1-amino-1-phthalidylpropane hydrochloride on excised and intact intestine and uterus** CHARLES M GRUBER and GOLDIE FREEDMAN KEYSER (by invitation) *Department of Pharmacology, Jefferson Medical College, Philadelphia, Pa*. In this study excised rabbit intestine was used as well as the intact intestine of non-anesthetized dogs with Thiry-Vella fistulae. Excised rabbit as well as intact rabbit, cat and dog uteri were also used. Racemate A and B of 1-amino-1-phthalidylpropane hydrochloride decreased the general tonus of freshly excised rabbit intestine in 60 per cent of the experiments. The intestine kept in a refrigerator for 24 hours responded to the drug by a decrease in tone in 91 per cent of the experiments. An increase in the general tonus was noted in the remaining experiments. The effects of histamine acid phosphate, acetylcholine chloride and pilocarpine nitrate on the excised rabbit intestine could be antagonized by 1-amino-1-phthalidylpropane hydrochloride. In five of the six non anesthetized dogs with Thiry-Vella loops, the intravenous administration of 1-amino-1-phthalidylpropane hydrochloride caused an increase in the general tonus of the gut. In the remaining animal 8 injections of the drug were

followed in each instance by a decrease in the general tonus. The increased tonus caused by the drug can be antagonized by either barbiturates, or thiobarbiturates. Excised rabbit uterine segments respond to 1-amino-1-phthalidylpropane by a sudden increase in the general tonus. Histamine acid phosphate, acetylcholine chloride or pituitrin, when added to the bath further increased the contraction of the uterus. The results on the intact uterus were variable. In dogs only contraction of the uterus was noted, but in cats and rabbits both decreases and increases in the general tonus were recorded.

**The oxygen content of coronary venous blood as affected by anoxia and cytochrome C** JOSEPH H HAFKENSCHIEL and JAMES E ECKENHOFF (introduced by CARL F SCHMIDT) *Department of Pharmacology and Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania*. Utilizing the bubble flowmeter and the catheterization technic of investigating the coronary circulation, a study has been made of the normal oxygen content of coronary venous blood and of the effect upon it of anoxia and of intravenously injected cytochrome C. In the experiments where the bubble flowmeter was used, coronary venous blood was obtained from a cannulated great cardiac vein, where the catheterization technic was used, coronary venous blood was withdrawn from the coronary sinus. In 20 control experiments, coronary venous blood contained 3-6 volumes per cent of oxygen when arterial blood contained 16.5 to 19.0 vol %. In a series of 5 other dogs, the average control oxygen content was arterial 19.8 vol % and coronary venous 6.5 vol %. With the inhalation of 8% oxygen these values became 9.5 vol % and 2.0 vol %. Cytochrome C was injected intravenously, in amounts varying from 3.8 to 12.5 mgm/kg, into 8 dogs made anoxic by breathing 8 to 14 per cent oxygen mixtures. In 7 instances there was no evidence of an increased removal of oxygen from the coronary blood. In one dog, the A-V oxygen difference was increased but only because the arterial oxygen content was increased over the control values.

**Toxicity of O,O-diethyl O-p-nitrophenyl thiophosphate (parathion)** ERNEST C HAGAN and GEOFFREY WOODARD (introduced by ARNOLD J LEHMAN) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*. Increasing interest in the insecticidal properties of O, O-diethyl O-p-nitrophenyl thiophosphate (parathion) led us to study its action on warm-blooded animals. The oral LD50's of this compound administered in corn oil solution are mice, 25 mg/kg, guinea pigs, 32 mg/kg, male rats, 30 mg/kg, female rats, 3 mg/kg. This sex difference in rats was not

observed when the compound was administered intravenously. The effects of oral or parenteral administration were lacrimation, salivation, intestinal hypermotility, and generalized fibrillary tremors. Deaths usually occurred in 24 hours, and resulted from respiratory failure. Application of 0.5% concentrations in propylene glycol resulted in a miosis lasting over 8 hours which could be reversed within a few minutes by instillation of atropine. Prior administration of physostigmine (1 mg/kg intraperitoneally) diminished the symptoms and increased the survival time of rats given oral LD<sub>50</sub> doses of parathion. These observations suggest that parathion possesses anticholinesterase activity. Nicotine or atropine exerted some protective effects in animals poisoned by parathion. Daily administration of sublethal doses of parathion indicates cumulative effects.

**Chloroquine in human giardiasis.** ALVIN S. HAMBLY, JR. (introduced by Hamilton H. Anderson) *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, California* (read by title). Quinaerine [3-chloro-7-methoxy-9-(1-methyl-4-diethylamino)acridine dihydrochloride] has been used with some success in control of intestinal giardiasis (*G. lamblia*) in man. Since chloroquine [7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline diphosphate], which is chemically related, has proved more active against malaria, trial in human giardiasis seemed indicated. Twenty-six patients who harbored *G. lamblia* were divided into two groups, 14 were given chloroquine and 12 were not treated. The oral dose was 2.2 mgm per kilo given daily for 7 days. Three of the 14 treated patients remained clear of infection during a 3 month follow up period during which an average of 12 or more stool specimens were examined. Half of those treated were freed of diarrhea during or immediately after therapy. The untreated controls continued to harbor giardia throughout the follow up period. Among treated individuals the drug caused no change in tests of urine, blood, liver, kidney or cardiac function, nor other objective evidence of intolerance. These observations do not confirm the report of Basnuevo and Sotolongo (Rev de Med Trop y Parasit, Bact, Clin y Lab, 12:113, 1946) who "cured" 12 of 15 patients with giardiasis using chloroquine. The dose schedule of these workers was 1.0 gm the first day, followed by 0.5 gm for 2 to 4 days in adults.

**Some extra-cardiac effects of digitalis.** CARROLL A. HANDLER and MARGUERITE LaFORGE (by invitation) *Dept of Pharmacology, Baylor Univ College of Medicine, Houston*. We have recently observed (Jour Pharmacol, 89:97, 1947) that the administration of non-toxic doses of

digitalis to dogs results in prominent changes in the fluid compartments of the body. Further studies on the extra cardiac effects of digitalis have revealed that a considerable elevation in blood potassium occurs concomitant with the shift in body fluids. The magnitude of these various effects were as follows: increase in "extracellular" fluid, 8-20%, decrease in plasma volume, 15-30% (checked by plasma protein determination), increase in blood potassium, 50-150%. The maximum changes occurred in 4-8 hrs. The potassium changes seemed to persist longer than the effects on body fluids.

**Action of peroxides on endameba histolytica.** EDER L. HANSEN and RACHAEL K. REED (introduced by Hamilton H. Anderson) *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, California*. The requirement of *E. histolytica* for a high reduction potential in its environment is of interest and prompted the investigation of the action of peroxides as amebacidal agents. The following compounds were tested in liver proteose peptone medium (Parasitology, in press), 48 hours exposure at 37°C.

|                              | Greatest dilution amebacidal                     |
|------------------------------|--|
| Superoxol                    | 1/1000 by volume                                 |
| Tertiary butyl hydroperoxide | 1/50 000   |
|                              | Greatest concentration tested no effect on ameba |
| Tetra ethyl phosphate†       | 1/10   |
| Urea peroxide                | 1/2500   |
| Sod. pyrophosphate peroxide  | 1/2500   |

\* Fungicidal activity reported by Combes (N Y State J Med 37:1927-1937)

† Peroxide nature not definitely proved

The amebacidal activity of tertiary butyl hydroperoxide is believed to be due to its stability which is greater than that of the other peroxides tested. This compound was given to naturally infected macaques at 100 mgm/kg daily for 10 days in gelatine capsules orally. During an 11-16 day post-treatment period, the iron-hematoxylin stained cysts appeared unusually vacuolated, and the nuclei were fragmentary. Ten days later the amebae regained their normal appearance. Non-pathogenic amebae did not show morphologic changes. The monkeys exhibited no apparent toxicity, either local or systemic.

**Functional analysis of the chronic neurotoxic action of streptomycin.** JOSEPH E. HAWKINS, JR. and WALTER J. O'SHAUGHNESSY (by invitation) *Merck Institute for Therapeutic Research, Rahway, New Jersey*. The earliest signs of cumulative intoxication

tion in cats receiving repeated doses of streptomycin subcutaneously are copious salivation and slight ataxia. Salivation, which usually becomes established after several days, appears to involve a conditioned response. Ataxia, at first confined to the hindlimbs, increases in severity with continued dosing and is accompanied by a progressive loss of the nystagmus evoked by rotation, affecting first the post-rotational and later the per-rotational response (Fed Proc, 6 125, 1947). Similar effects have been produced with all salts of streptomycin tested, but the time of onset may vary with the purity of the preparation, of which the microbiologically determined chemotherapeutic potency is not necessarily a complete measure. Thus when the hydrochloride (potency 668 units per mg) was administered to 4 cats in daily doses of 100 mg base per kg body weight, 13 to 24 doses (mean 19) were given before symptoms appeared, while in 4 cats treated with equivalent doses of the crystalline calcium chloride complex (potency 655 units per mg) 32 to 51 doses (mean 38) were required. When the drug is withdrawn, the ataxia and other manifestations of vestibular disturbance gradually disappear. After a few months only a slight awkwardness remains, but little or no recovery of nystagmus occurs in cases of severe loss. Comparison with the results of labyrinthectomy suggests that impairment of peripheral vestibular mechanisms alone is insufficient to account for the neurotoxic effects. Certain closely related central vestibular, cerebellar and optomotor functions appear also to be affected by streptomycin.

**Pharmacological actions of O-O-diethyl O-p-nitrophenyl thiophosphate** LLOYD W. HAZLETON and EMILY GODFREY (by invitation) *Hazleton Laboratories, Falls Church, Virginia*. This compound is also known as Thiophos 3422 Insecticide. The most prominent actions of diethyl p-nitrophenyl thiophosphate are muscarinic, but these are not complete and there is evidence of nicotinic action as well. In rabbits dermal application produces diarrhea in lethal or nonlethal doses and there is some salivation. Myosis is not prominent following systemic administration, but application to the eye produces hyperemia and maximal constriction with return to normal overnight. This action is not reversed by atropine but may be prevented by prior administration. In the anesthetized dog intravenous administration of diethyl p-nitrophenyl thiophosphate is followed in eight to ten minutes by increased pulse pressure and decreased cardiac rate, accompanied by decreased respiratory volume with little change in rate. At the extreme, when respiration is limited to inspiratory spasm, both cardiac and respiratory effects may be almost completely relieved by 1 mg/kg of atropine sulfate. Atropine given prior to oral administration of diethyl p-nitrophenyl

thiophosphate exerts an appreciable life saving effect in rats and mice. Atropine does not antagonize the peripheral muscular spasms which appear to be nicotinic in nature.

The action of p-dimethylaminobenzenediazo sodium sulfonate on carbohydrate metabolism. ROY G. HERRMANN (by invitation) and KENNETH P. DUBOIS. *University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago*. Read by title. p-Dimethylaminobenzenediazo sodium sulfonate (DAS) has been employed as a rodenticide in Germany. This use together with the chemical similarity of DAS to the carcinogenic azo dyes stimulated our interest in the mechanism of action of this compound. The approximate LD<sub>50</sub> of DAS to rats was 15 mg/kg. After uniformly lethal doses symptoms appeared in about 2 hours and consisted of depression, flaccidity, and terminal convulsions. After 30 mg/kg of DAS intraperitoneally to rats, which resulted in death in 3 to 8 hours, a rise in blood glucose from an average normal value of 94 mg% to 172 mg% occurred in 3 hours followed by a decrease to 20 mg% at the time of death. Liver glycogen decreased from 2.14% to 0.114% in 5 hours after 30 mg/kg of DAS. The administration of glucose prevented the terminal convulsions and insulin and adrenal-demedullation counteracted the hyperglycemia but did not prevent death. DAS inhibited the oxidation of glucose by rat liver slices *in vivo* and *in vitro*. A final concentration of  $1 \times 10^{-4}$  M DAS produced 53% inhibition of the oxidation of glucose by liver slices and at 3 hours after 30 mg/kg of DAS a 38% decrease in the oxygen consumption of liver slices was noted. Enzyme studies have indicated that DAS inhibits an oxidative enzyme other than succinic dehydrogenase, cytochrome oxidase, or malic dehydrogenase since no decrease in the activity of these enzymes was observed in livers from rats poisoned by 30 mg/kg of DAS.

The water-soluble B-glucoside of desoxycorticosterone acetate, its adrenal cortical activity in adrenalectomized dogs. E. HERROLD (by invitation), H. HAYS (by invitation), G. HOLMQUIST (by invitation), B. RICHARDS (by invitation), E. OPPENHEIMER. *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey*. Initial experiments have confirmed Meier's work (Meier, R., Gysel, H. and Mueller, R., *Schw. med. Wschr.* 74 93, 1944) on the doses of the B-glucoside of Desoxycorticosterone Acetate necessary (1) to maintain an adrenalectomized dog, (2) to correct the syndrome of severe adrenal insufficiency. On a molar basis, 1.6 times as much glucoside as DCA in oil was required to maintain an adrenalectomized dog previously supported with DCA in oil. Meier reported that twice the dose of glucoside, on a molar basis, pro-

vided maintenance but that 1.5 times the dose of DCA in oil did not prevent slight symptoms of insufficiency. In our experiments, the dogs were first "normalized" after operation by minimal doses of DCA in oil. We found that 0.35 mg of DCA glucoside/kg of body weight maintained the animal, Meier recommends 0.4 to 0.6 mg/kg. Symptoms of severe adrenal insufficiency appeared within 24 to 72 hours after withdrawal of the glucoside and once initiated, progressed with unique speed. Meier has recommended from 16 to 50 fold the normal maintenance dose to correct severe adrenal insufficiency, and our experiments have confirmed this range. Because the water-soluble B glucoside of DCA can be administered intravenously, its corrective action in severe adrenal insufficiency is rapid and dramatic.

The effects of 2,4 dinitrophenol and thiouracil on chronic methanol poisoning. CHARLES H. HINE and THOMAS NATHANIEL BURBRIDGE (by invitation). *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*. Fifty rats of the Long-Evans strain were divided equally as to sex and weight (average 225 gm.) into 5 groups. The first group received methanol, the second, methanol plus dinitrophenol, the third, methanol plus thiouracil, fourth, dinitrophenol, and the fifth, thiouracil. These were given for the life of the animal up to 120 days. Seven per cent methanol, ingested as the only source of fluid, caused mortality in all animals over a period of 60 days. The mean survival time was 43 days and the mean weight loss was 99 grams. Simultaneous ingestion of 0.4% of 2,4 dinitrophenol increased the mean survival time significantly (76 days) in a second group of 10 rats but did not lower the mortality. Use of 0.2% thiouracil further increased the survival time (87 days) and decreased the mortality, 4 of 10 survived. The weight loss of the second group was greater and that of the third group less than noted in rats receiving methanol alone. Mean survival time was not affected (78 days) when 0.4% of 2,4 dinitrophenol was ingested alone (group four), but was increased (120 days—to end of observation) with 0.2% thiouracil (group five). There was a steady weight gain in group five for the first 100 days, after which weight loss occurred. All other groups showed an initial and progressive loss of weight. Bile staining of the liver, kidney, spleen, gut, and occasionally of the heart and lung was noted at autopsy in all rats receiving methanol (in the first three groups). Staining was most pronounced in those animals given methanol alone and least marked in those receiving thiouracil plus methanol. All groups which received methanol also showed an increased urine acidity

(pH 1.0) and a slightly decreased visual perception of light.

The comparative activity of Some  $\alpha$  substituted glycerol and glycidyl ethers. CHARLES H. HINE and HERBERT E. CHRISTENSEN (by invitation). *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, California*. The comparative toxicity of a series of  $\alpha$  substituted glycerol and glycidyl ethers has been determined in mice (30 animals for each agent) following oral and subcutaneous administration. These compounds have a curare like action and produce loss of the righting reflex, muscular flaccidity and death through respiratory paralysis. The glycidyl compounds are generally more potent than their corresponding glycerol derivative. Replacement of the hydroxy groups of the  $\beta$  and  $\gamma$  carbons by one or more aliphatic groups decreased the potency. Delayed death occurred with most of the compounds following 24 hour exposure to the saturated vapors. Percutaneous absorption caused death with only the more toxic derivatives, such as glycidyl phenyl ether and glycidyl isopropyl ether. There was no irritating effect following topical application of the aliphatic compounds but the aromatic derivatives produced irritation and a leathery induration in the unabrased shaved skin of albino rabbits. The LD<sub>50</sub> for both species was within the same range for any one of the compounds tested. Rabbits were slightly more resistant to oral administration. Neck drop was elicited in this species as an early sign of muscular paralysis after safe as well as fatal doses.

Certain aspects of the acute toxicity of beryllium following intraperitoneal injection. HAROLD C. HODGE (by invitation), ELLIOTT A. MAYNARD (by invitation), and WILLIAM L. DOWNS (by invitation). Introduced by HARVEY B. HAAG. Following intraperitoneal injection in rats and mice, the LD<sub>50</sub> dose based on 24-hour mortalities was determined for various beryllium compounds. A few rabbits and guinea pigs were also injected. The soluble beryllium salts gave indication of toxicity of varying degree, the insoluble salts were practically non-toxic. Saline solutions were less toxic than aqueous solutions. Young (weanling) rats and mice were more resistant to beryllium toxicity than were older animals.

Two new analgesics. HELEN L. HOLLAND (by invitation) and E. G. GROSS. *Department of Pharmacology, School of Medicine, Iowa City, Iowa*. The *Cis* (Nu 1196) and *Trans* (Nu 1779) forms of 1,3-Dimethyl-4-phenyl-4-propionyloxy piperidine hydrochloride have been compared with morphine for analgesic potency in normal human subjects and in dogs, using the Wolff-Hardy technique. The analgesic potency of the *Cis* form in dogs is slightly greater than morphine, while the *Trans*

form is about 4 to 5X as potent as morphine. In man, the Cis form is somewhat less analgesic and the Trans form about equal in analgesia potency to morphine. The duration of analgesia of the Cis form is shorter and that of the Trans form about the same as morphine. Mild sedation is produced by the Cis form and rather marked sedation by the Trans form. Untoward effects of the Cis form are relatively few, only slight dizziness, minor itching and little or no nausea in doses up to 15 mgs. The Trans form produces marked dizziness, sweating, itching, nausea and vomiting are frequent at the 10 and 15 mg level. No significant alterations in blood pressure or pulse rates have been observed with either form. No analgesic tolerance or alteration in RBC or WBC was observed in dogs receiving daily injections for 6 weeks.

A comparison of the toxicity of methadon and some related compounds. By JAMES O. HOPPE (by invitation) and LLOYD C. MILLER. *From Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N. Y.* Acute toxicity studies in both mice and rats and subacute toxicity studies by subcutaneous administration to rats were made using aqueous solutions of the hydrochlorides of methadon, 6 dimethylamino-4,4-diphenyl-3-heptanone, its levo isomer, the *dl* form of a structural isomer, 6 dimethylamino-4,4-diphenyl-5-methyl-3-hexanone (Win 1783), ethyl-1-methyl-1-(*m*-hydroxyphenyl)-piperidine-4-carboxylate (Win 771), and 1-methyl-4-(3-hydroxyphenyl)-4-piperidyl ethyl ketone (Win 1539). The following acute LD<sub>50</sub> values (mg per kg) for mice were found:

|               | Intravenous | Subcutaneous | Oral     |
|---------------|-------------|--------------|----------|
| methadon      | 13 ± 1      | 18 ± 4       | 141 ± 0  |
| Levo-methadon | 13 ± 1      | 15 ± 3       | 111 ± 12 |
| Win 1783      | 22 ± 2      | 40 ± 5       | 136 ± 8  |
| Win 771       | 54 ± 2      | 332 ± 59     | 771 ± 41 |
| Win 1539      | 27 ± 6      | 18 ± 5       | 120 ± 23 |

The dose-mortality curves by subcutaneous administration were less steep than by either the oral or intravenous routes for each compound except Win 1539 which showed flat curves by all three routes. Thus doses of Win 1539 four times greater than those lethal to some mice failed to produce 100% mortality. Also this compound gave such erratic results by parenteral administration that the apparently greater toxicity by the subcutaneous route is insignificant. Subacute toxicity determinations were made in rats by daily subcutaneous administration for three weeks. The incidence of toxicological manifestations, particularly the inhibition of gain in body weight in the methadon series, was least with Win 1783 and greatest with levo-methadon. Win 1539 by this test

was less toxic than levo methadon, but more toxic than Win 1783.

Opposite effects in vitro and in vivo of a surface active agent on mycobacterium tuberculosis. S. H. HOPPE (by invitation), V. V. COLE, H. R. HULPIEV, and H. A. RAIDT (by invitation). *Depts of Public Health, Pharmacology, and Microbiology, Indiana University School of Medicine, Indianapolis, Ind.* The agent used, Igepal, is described as a polyethylene alcohol condensate. The growth of Mycobacterium tuberculosis (H37 strain) was studied on Dubos' synthetic medium with and without albumin, and on beef infusion agar. When Igepal was added to the media there was complete inhibition of growth with concentrations of 0.005 per cent and above on Dubos' media and at 0.01 per cent and above on beef infusion agar. Guinea pigs were infected with human strains of tuberculosis and when more than half showed evidence of local involvement they were placed on the experimental diet. In a group of four receiving 0.5 per cent Igepal, all were dead 80 days later, whereas only one control was dead. The Igepal pigs all showed tuberculous involvement of the liver, while controls showed none. The spleens were larger in the Igepal pigs and showed a more widespread conglomerate type of tuberculosis. This experiment was repeated with a more virulent strain of the tubercle bacillus and 0.1 per cent Igepal in the diet. Eighty days after being put on the diet 3 of 13 controls and 9 of 12 Igepal pigs were dead. Organs involved were about the same in the two groups. There was no difference in spleen size but there was more of a conglomerate type of tuberculosis in the Igepal pigs. The results with 0.1 per cent Igepal were less marked than with 0.5 per cent but in the same direction. In vitro studies gave opposite results.

Procaine metabolism in dogs anesthetized by thiopental, ether and chloroform. H. R. HULPIEV, and V. V. COLE. *Dept of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis, Ind.* Procaine HCl was given intravenously at various constant rates to dogs under light anesthesia (plane 1). Dogs under sodium thiopental withstood considerably larger doses and lived longer than those under chloroform. Dogs under chloroform were more resistant than those under ether. At death the blood levels of procaine were approximately the same under thiopental as under chloroform but under ether they were about 60 per cent lower. The brains of the dogs under thiopental had a higher concentration of both procaine and *p*-aminobenzoic acid than those under chloroform or ether. The livers from thiopental dogs had a lower concentration of procaine but a higher concentration of PABA than those from ether and chloroform dogs. The same concentration of procaine was found in the kidneys

of dogs under thiopental and chloroform but approximately 10 per cent less was found in the kidneys of these dogs given ether. PABA concentration in the kidneys was greatest under thiopental and least under ether. A time factor undoubtedly played a considerable part in the above distribution. Conversion of procaine to PABA was most rapid under thiopental and slowest under ether. For any given dose of procaine the ratio of blood PABA to that dose was largest under thiopental and smallest under ether. Under all three anesthetics the concentration of procaine was uniformly higher in the brains, livers and kidneys than in the blood. Concentration of PABA in livers and kidneys was higher than that in blood, while the concentration in the brains was relatively low.

**Structural relationship to sympatholytic activity of certain B-chlorethyl amines** CARLTON C HUNT (introduced by McKee Cattell) *Department of Pharmacology, Cornell Univ. Medical College and Sloan-Kettering Institute, New York, N. Y.* B-chlorethyl amines of the following structure

$$\text{R}-\text{N}-\text{CH}_2\text{CH}_2\text{Cl}$$

were tested for a protective

effect against lethal doses of epinephrine (LD 80-100) in mice. These compounds were injected subcutaneously (20 mg./kg.) 30 minutes prior to the intraperitoneal administration of epinephrine. Effective protective agents were injected in progressively smaller doses until 10% or more animals died after epinephrine. The following substitutions offered protection (minimum protective dose mg./kg. in parenthesis): R and R' = benzyl (10), p-methoxybenzyl (10), and m-methylbenzyl (5), R, R' = benzimidazolomethyl, benzyl (10), a naphthylmethyl, ethyl (0.1), a naphthylmethyl, B-chlorethyl (0.5), benzyl, B-chlorethyl (0.5). Dimethyl B-chlor, B-phenylethyl amine was also effective (1 mgm./kgm.) whereas dibenzyl B-chlor, B-phenylethyl amine was inactive. The following substitution on both benzyl groups of dibenzyl B-chlorethyl amine (dibenamine) abolished protective activity: p-isopropyl, o-Cl, o-methyl. Substitution of p-nitro, o-Cl, and p-Cl on benzyl bis(B-chlorethyl) amine abolished activity. As previously reported substitution of the phenyl group directly to the N lacks activity.  $\gamma$ -phenyl propyl bis(B-chlorethyl) amine is inactive. Effective protective compounds were tested on the blood pressure response (cat) to injected epinephrine and acetylcholine (atropinized animal). In all cases the pressor responses were abolished. Benzyl bis(B-chlorethyl) amine and a naphthyl methyl bis(B-chlorethyl) amine also prevented the fall in blood pressure following injection of epinephrine or isuprel. Sympatholytic activity appears to depend upon a phenyl or naphthyl group

attached to N through 1 or 2 carbons, the N being attached to 1 B-chlorethyl and another alkyl or aryl group. As reported by Loew et al. a biphenoxyethyl derivative is also active.

**Sudden deaths during chloroform and cyclopropane anaesthesia** DUNCAN E. HUTCHESON (introduced by G. H. W. Lucas) *Department of Pharmacology, University of Toronto* Guinea pigs, cats and dogs have been used to investigate the mechanisms causing sudden deaths under chloroform and cyclopropane. Changes in the cardiac rate and rhythm were observed during all stages of anaesthesia and after the injection of small doses of epinephrine. Under chloroform, 9 of 22 guinea pigs and 3 of 10 cats died abruptly as the result of ventricular fibrillation following the injection of epinephrine, the same dose of epinephrine produced no deaths in an equal number of animals. All cats except those that died of ventricular fibrillation developed a transient tachycardia following the epinephrine. Five of 10 dogs under chloroform and 3 of 7 dogs under cyclopropane died of epinephrine induced ventricular fibrillation. Deaths occurred during light anaesthesia with chloroform and during deep anaesthesia with cyclopropane. The same dose of epinephrine causes a reflex bradycardia in the normal unanaesthetized dog and under ether, so that chloroform and cyclopropane alter the reaction of the heart to epinephrine. Chloroform and cyclopropane may increase the irritability of the myocardium or may decrease reflex vagal activity. In rats and rabbits ventricular fibrillation could not be produced by epinephrine under either chloroform or cyclopropane.

**The effect of curare on the esophagus of the dog** K. HWANG (by invitation) and K. R. UNRA *Department of Pharmacology, University of Illinois College of Medicine, Chicago 12, Illinois* The order in which individual muscles are affected by curare appears to be a function of the physiologic characteristics of the respective muscle. Reports in the literature regarding the effect of curare on the striated musculature of the esophagus are conflicting. The present work was designed to study the effect of d-tubocurarine on the cervical and thoracic portions of the esophagus which, in the dog, receive separate motor innervation (Hwang, Grossman and Ivy, Fed. Proc. 6: 133, 1947). In dogs anesthetized with pentobarbital, contractions of the leg muscle and of the two portions of the esophagus were recorded. Their respective motor nerves were stimulated with condenser discharges of various frequencies for specified periods of time. Tetanic contractions of the esophagus were not sustained at stimulation rates of more than 50 per second. On the other hand, the leg muscle showed well sustained tetanus on stimulation at rates of more than 100 per second. Small amounts of

d-tubocurarine depressed the maximum rate of stimuli which were capable of causing sustained tetanus of the muscle. This effect occurred almost simultaneously in the leg muscle and in the different portions of the esophagus. D-tubocurarine paralyzed skeletal muscle and both portions of the esophagus before arresting the contractions of the diaphragm. Recovery of the diaphragm was followed by recovery of the intercostal and other muscles. However, the time for the full recovery of the esophagus was four or five times longer than that of the diaphragm.

**The baljet reaction and pharmacodynamic studies of diginin and gitogenin.** HARRY K. IWAMOTO and FREDERICK K. BELL (introduced by JOHN C. KRANTZ, JR.) *Department of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.* Recently a chemical method for the assay of digitalis preparations was described. This method utilizes the Baljet reaction in which a red orange color is developed by the active glycosides which is proportional to their cardiotonic activity. Diginin, a glycoside isolated from *digitalis purpurea*, was found to exhibit a weak Baljet reaction and little cardiac action. Gitogenin, a sapogenin, was found to be inactive on the heart and gave a negative Baljet test. Since each of these substances is obtainable in significant yields along with the cardiotonic principles from *digitalis purpurea*, the importance of these properties with respect to the chemical assay is apparent.

**Influence of some analgetics of obstetrical importance upon respiration of mature human placenta *in vitro*.** HAL P. JAMES and ERNEST W. PAGE (introduced by C. H. HINE) *Divisions of Obstetrics and Gynecology and of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco* (read by title). Studies of the effects of various analgetic drugs upon the respiration of rat cerebral cortex slices have been reported by Fuhrman and Field (*J. Pharmacol.*, 77: 392, 1943) and by Elliott, Warrens and James (*J. Pharmacol.*, 91: 98, 1947). Inasmuch as the placenta is the only human tissue that may be conveniently studied *in vitro*, we wished to compare the influence of similar drugs upon this tissue. For this purpose, we have selected those agents commonly used for pain relief in labor. The influence of merperidine (demerol), methadon ("10820"), morphine, amylal and scopolamine upon the rate of oxygen consumption of placental tissue was studied by the direct method of Warburg. The fresh tissue was prepared in the cold by teasing the villi apart with blunt dissection. Approximately 200 mgm. samples were suspended in modified Krebs-glucose-ringer-phosphate solution. The oxygen uptake was determined at 20-minute intervals for 80 minutes before and

after addition of the drug. The results are summarized below.

| Drug  | Concentration | Max. effect on QO  | Time of max. effect |
|---|---------------|--------------------|---------------------|
|   | moles/liter   | % of control value | minutes             |
| Demerol (merperidine)                                 | 0.00025       | -10                | 20                  |
|   | 0.0005        | -27                | 20                  |
|   | 0.001         | -43                | 20                  |
|   | 0.0075        | -83                | 40                  |
| Methadon (10820) <small>10820 done, dolophine</small> | 0.00025       | +22                | 60                  |
|   | 0.0005        | +33                | 60                  |
|   | 0.001         | +12                | 40                  |
|   | 0.002         | -93                | 120                 |
| Morphine  | 0.00025       | no effect          |                     |
|   | 0.005         |                    |                     |
| Amytal  | 0.0001        | +1                 | 20                  |
|   | 0.0002        | -9                 | 60                  |
|   | 0.00045       | -31                | 20                  |
|   | 0.0009        | -63                | 80                  |
|   | 0.0018        | -97                | 120                 |
| Scopolamine   | 0.00025       | -11                | 20                  |
|   | 0.005         | -21                | 20                  |
|   | 0.02          | -56                | 20                  |

Demerol, amylal and scopolamine appear to be true depressants, whereas morphine has little, if any, effect. Methadon stimulated respiration in low concentrations, but depressed it at higher levels. All concentrations used are considerably in excess of therapeutic, and the results do not correlate with effects of the same drugs upon the intact fetus. They do, however, correspond well with the above-mentioned studies on rat cerebral cortex.

**Experimental bromism in man.** E. M. JELLINEK, *Dept. of Applied Physiology, Yale University* (introduced by C. I. BLISS). In testing clinical reports on the occurrence of bromide psychosis at blood bromide levels ranging from 50 to 120 mg/100 cc, 78 psychologically normal persons and 60 psychotic patients were kept for several weeks at blood bromide levels around 200 mg/100 cc. Subjective and objective effects of the bromide administration were observed. A psychological test battery was employed to check on subjective reports. The statistical analysis takes account of the practice factors involved in these tests and attempts to arrive at the net effect of the drug. Even at fairly high bromide levels the normal subjects did not show gross psychological or neurological effects but only manifestations of sedation. The indications are that bromide psychosis at bromide levels reported in the clinical literature would occur largely in individuals with psychotic liabilities or at the onset of a psychosis.



Comparative analgesic and toxic effects of the optical isomers of methadon and isomethadon. ELIZABETH H. JENNEY (by invitation) and CARL C. PFLIFFER, *Department of Pharmacology, University of Illinois College of Medicine, Chicago 12*. Acute toxicity was determined by intraperitoneal administration of these drugs using the albino mouse (Harlan strain, 18-24 Grams, both sexes). With both drugs the d optical isomers were more convulsant and produced a less marked Straub-tail phenomenon. Analgesia was determined after subcutaneous administration in the guinea pig (method of Winder, Pfeiffer and Maison). An abnormally low therapeutic index was derived as a ratio of the mouse LD-50 to the minimal anal-

|                | Mouse<br>LD 50 | S.E.    | Slope | Minimal<br>analg.<br>dose | Therap.<br>index |
|----------------|----------------|---------|-------|---------------------------|------------------|
| dl Methadon    | 29             | $\pm 2$ | 11    | 12.5                      | 2.3              |
| dl Isomethadon | 39             | $\pm 3$ | 9     | 12.5                      | 3.1              |
| l Methadon     | 11             | $\pm 2$ | 11    | 5                         | 2.2              |
| d Methadon     | 63             | $\pm 3$ | 14    | 20                        | 3.2              |
| l Isomethadon  | 25             | $\pm 2$ | 7     | 7.5                       | 3.3              |
| d Isomethadon  | 73             | $\pm 6$ | 9     | 30                        | 2.4              |

\* All doses are in mgm /kgm

gesic dose in the guinea pig. From these data dl Methadon has a slightly higher therapeutic index which is due entirely to a higher LD 50. Chronic toxicity studies in weanling rats (Sprague Dawley strain, both sexes) were made using a dose one-third that of the mouse LD 50. Injections were made subcutaneously daily for a period of five weeks. These data indicate that judged by the criteria of mortality, weight loss, and blood changes, d Methadon is the least chronically toxic and d Isomethadon is the most chronically toxic, while the two l Isomers are intermediate. The data further indicate the need for clinical studies on the pure l Isomethadon rather than the racemic mixture.

The relationship of cholinesterase inhibiting activity to the acute toxicity of some organic phosphorus compounds. H. WALTER JONES, JR. (by invitation), BERTRAM J. MEYER (by invitation) and LEONARD KAREL, *Toxicology Section, Medical Division, Army Chemical Center, Maryland*. The degree of *in vitro* inhibition of rat brain cholinesterase caused by 42 organic phosphorus compounds was compared with their acute (24 hour observation period) intraperitoneal toxicity to mice. The 38 compounds which proved to be weak inhibitors of cholinesterase (50% inhibition caused by molar concentrations greater than  $2.5 \times 10^{-4}$ ) were also comparatively non toxic (LD<sub>50</sub>'s greater than 425 micromols/kg or 100 mg/kg), and no correlation between cholinesterase inhibition and toxicity was observed in this group. The remaining

four compounds were potent inhibitors of cholinesterase. These were (1) tetraethyl pyrophosphate, (2) diisopropyl fluorophosphate, (3) diisopropyl chlorophosphate, and (4) diethyl p chlorobenzene phosphonate. Two other potent cholinesterase inhibitors (A) and (B) (commercial confidential) were also investigated. The concentrations in which these six compounds caused 50% inhibition of cholinesterase activity and their LD<sub>50</sub>'s in micromols/kg and mg/kg are presented in tabular form below.

| Cmpd | M Conc. for 50%<br>inhib | LD <sub>50</sub> | LD <sub>50</sub> |
|------|--------------------------|------------------|------------------|
|      |                          | micro M/kg       | mg/kg            |
| 1    | $0.3 \times 10^{-3}$     | 4.00             | 1.16             |
| A    | $1.0 \times 10^{-3}$     | 9.48             | 3.49             |
| 2    | $4.3 \times 10^{-7}$     | 47.5             | 8.74             |
| B    | $4.2 \times 10^{-7}$     | 58.8             | 10.4             |
| 3    | $2.0 \times 10^{-4}$     | 146              | 29.2             |
| 4    | $1.6 \times 10^{-5}$     | 1120             | 300              |

When the logs of these LD<sub>50</sub>'s in micromols were plotted against the negative logs of the molar concentrations causing 50% inhibition, a straight line was obtained. Thus the toxicity of these compounds appears to be a function of their potency as cholinesterase inhibitors.

Action of central nervous system depressants at different growth periods of salamander (*Ambystoma punctatum*) larvae. ALEXANDER G. KARCZMAR (by invitation) and THEODORE KOPFANYI, *Department of Pharmacology and Materia Medica, Georgetown University School of Medicine*. During the course of studies on the correlation of drug action and differentiation of the nervous system of *Ambystoma punctatum*, eight depressants were employed. Larvae of different ages (from 10 to 33 mm in length) were immersed in standard concentrations of each of the depressants, and the anesthetic induction period (time in minutes elapsing between the immersion of larvae in solution and the onset of full anesthesia) recorded and plotted against larval lengths. For paraldehyde (1:200), ethyl alcohol (1:33), and tricain (MS 222, 1:7,500) the induction period was shortened gradually from a value of 40 to 30 to about 10 minutes, with a possible break in the curve occurring at larval length of 24 mm. It decreased abruptly for sodium barbital (1:20), nembutal (1:250), chloral hydrate (1:250) and chloretone (1:4,000), 12 mm long larvae requiring more than two hours for development of full anesthesia, while 33 mm long larvae were narcotized within from 5 to 10 minutes by chloretone and chloral hydrate and within 30 minutes by the two barbiturates. A possible break in the curve occurred at an early growth stage for chloretone, and at an

intermediate stage for the barbiturates and chloral hydrate. Finally, a 1:1,000 solution of acetanilid anesthetized the larvae within 6 to 15 minutes irrespective of larval length. Also, the minimal effective concentration of acetanilid (1:1,500) did not vary within the range of the larval age investigated, while the minimal effective concentrations of other depressants studied became progressively lower as the larval growth proceeded.

**A potential rodenticide—"Fanyline"** LLOYD KAREL *Toxicology Section, Medical Division, Army Chemical Center, Maryland*. Subsequent to the report of Kalmbach in 1946 on the effectiveness of sodium fluoroacetate, "1080", as a rodenticide, attempts were made to find an equally effective vermicide which was, unlike "1080", relatively water insoluble. Potentially such a compound has been found in fluoroacetphenylhydrazide, hereafter called "Fanyline". Oral toxicity was determined in non fasted animals by administering suspensions of "Fanyline" in 20% gum acacia through an oesophageal catheter or, in cats and dogs, by feeding capsules. Calculated median lethal doses were 9.1 mg/kg for male rats, 14.9 mg/kg for female mice, and 7.2 mg/kg for pigeons, whereas the estimated LD<sub>50</sub>'s were slightly less than 1.3 mg/kg for rabbits, between 0.65 and 1.0 for guinea pigs, greater than 0.25 and less than 0.5 for cats, and between 0.1 and 0.25 for dogs. Mixtures of 1% and of 2% "Fanyline" in corn meal were highly acceptable to albino rats and mice, well-fed rats consuming poisoned meal even when presented to them side by side with their normal diet. In acceptability tests, all of 32 rats weighing 200 to 500 gms and 16 rats weighing 65 to 82 gms ate enough of the "Fanyline" to cause death. Furthermore, despite the relative non-lethality of the compound for mice, of 100 animals comprising four equal groups exposed to 2% poisoned meal, 33 consumed lethal quantities without pre-baiting, while of the 67 survivors, 55 consumed lethal doses after being pre-baited for one week. Toxicity and acceptability tests on wild Norway and Alexandrine rats are currently in progress in other laboratories.

**Circulatory and adrenolytic actions of DHE and DHO** NORMAN W. KARR (introduced by NORMAN A. DAVID) *Department of Pharmacology, University of Oregon Medical School, Portland, Oregon*. Several hydrogenated ergot alkaloids have been shown to be less toxic and stimulant to smooth muscle than their naturally occurring precursors, while retaining full or augmented potency as adrenolytic agents (Rothlin, *Bull. Schweiz. Akad. Med. Wiss.*, 2:249, 1947). We have compared dihydroergotamine (DHE), dihydroergocornine (DHO) and ergotamine (Gynergen, Sandoz) with regard to reversal or diminution of pressor response to injected epinephrine, and alteration

produced in the blood flow to a hind limb as measured with a bubble flowmeter (Soskin et al., *Am. J. Physiol.*, 108:107, 1934). Dogs were anesthetized with intravenous sodium pentobarbital or with intragastric urethane and chlorobutanol. Under the conditions of our experiment, ergotamine has not consistently produced reversal or decrease in the pressor response to injected epinephrine, but in about 50% of the experiments a dose of 0.1 mg per kg has significantly diminished blood flow to the hind limb. This dose produces a marked rise in blood pressure, and frequently respiratory arrest and death. DHE and DHO in identical doses are well tolerated and without notable effects on blood pressure or respiration. Following either dihydrogenated alkaloid the pressor response to epinephrine is slight and transient and usually followed by a more marked and sustained fall in pressure. Neither DHE nor DHO has produced significant alterations in blood flow to the hind limb.

**Toxicity of intravenous ammonium salts** NORMAN W. KARR and EDWARD L. HENDRICKS (introduced by NORMAN A. DAVID) *Department of Pharmacology, University of Oregon Medical School, Portland, Oregon*. Intravenous ammonium chloride has been reported to be a valuable treatment for alkalosis or certain types of oliguria, particularly where oral use of acidifying agents is precluded. While reactions characterized by pallor, sweating, and retching have been observed even with low concentrations (Schemm, reported before Western Soc. Clin. Res., Nov. 1947), search of the literature fails to reveal any systematic study of the toxicity of intravenous ammonium compounds. We have given ammonium chloride, carbonate, and bicarbonate to dogs by intravenous infusion, and find that all produce the same typical chain of toxic signs. The first is the appearance of occasional gasps, which become more frequent until the respiration consists only of a series of jerking gasps, with intervening periods of apnea. Simultaneously the heart rate becomes very slow, and often irregular. Fibrillary twitchings of the tongue appear, frequently followed by generalized fibrillations and tonic convulsions. If administration is continued, death soon ensues. This sequence of toxic signs is closely correlated with the rate of administration of the ammonium ion, regardless of the salt used. It has appeared with rates as low as 0.105 milliequivalents per kg per minute, and a rate of 0.182 milliequivalents per kg per minute has produced toxic signs in 15 minutes. Clinically ammonium chloride has been used in 2% solution, at the rate of 500 ml per hour, which for a 70 kg man, amounts to 0.045 milliequivalents per kg per minute. Thus the safety margin for ammonium chloride given intravenously in this concentration is quite narrow,

ranging in 16 of our experiments from 2.29 to 4.37. This suggests the importance of close supervision of the rate of intravenous infusion of ammonium chloride, as double or treble the recommended rate might lead to serious toxic reactions.

Comparison of blood levels of thiopental (pento-thal), 5-allyl-5-(1-methylbutyl)-2-thiobarbituric acid (Surital) and thioethamyl in the dog. A. R. KELLY (by invitation), F. E. SHIDEMAN and B. J. ADAMS (by invitation) *Dept. of Pharmacology, Univ. of Michigan*. Using the Beckman spectrophotometer and a modification of the method of Jailer and Goldbaum, absorption and calibration curves and recoveries from plasma (96-100%) were obtained for three thiobarbiturates, thiopental, 5-allyl-5-(1-methylbutyl)-2-thiobarbituric acid (Surital), and Thioethamyl. Preliminary experiments demonstrated that withdrawal of blood from dogs in amounts up to 80 cc did not significantly alter plasma blanks. It was also found that incubation of thiopental in plasma for 3 hours at 38°C resulted in no appreciable diminution of recoverable drug. Blood level curves were obtained on dogs injected intravenously with sodium thiopental (20 mg/kg, 4% solution) and equimolar doses of Surital and Thioethamyl. The average duration of action and the blood level at the time of return of the righting reflex for each drug as follows: thiopental, 54 min, 1.2 mg/kg, Surital, 94 min, 0.86 mg/kg, Thioethamyl, 11 min, 2.1 mg/kg. A comparison of potency based on the above blood levels and using thiopental as a standard of 100%, indicates that Surital has a potency of 139% and Thioethamyl, 57%. The shape and slope of the blood level curves for all three drugs was similar, indicating approximately equal rates of detoxication. A peak of approximately 3.5 mg/kg was reached within one minute after completion of the injection, the level falling rapidly to 2.5 mg/kg at 3-5 minutes. At 20 minutes it had reached 1.5 mg/kg and leveled off so that at one hour the level was approximately 1 mg/kg and at 4 hours 0.5 mg/kg.

The anticholinergic activity of congo red and related compounds. CHARLES J. KENSLEY (introduced by McKEN CATELL) *Department of Pharmacology, Cornell University Medical College*. Congo red and related compounds have been found to possess prophylactic and therapeutic anticholinergic activity in the frog when pure di-tubocurarine chloride (DTC) is the curarizing agent. Two pure erythrina alkaloids, beta erythroidine (BE) and dihydro-beta erythroidine (HBE) have also been studied. In contrast to the anti-DTC activity of congo red, no antagonism of the curarizing action of the erythrina alkaloids was observed. Disopropyl fluorophosphate was less effective against DTC than congo red, but was as effective against BE as against DTC. Congo red is a moderately potent

cholinesterase inhibitor *in vitro* but its failure to protect against curarization by the erythrina alkaloids as well as the lack of anticholinesterase activity of chlorazol fast pink (a related compound which possesses anti-DTC activity) indicates that the anti-DTC activity of congo red is not due chiefly to its anticholinesterase activity. Evidence has been obtained which supports the hypothesis that the anti-DTC activity of congo red and related compounds is due to a reaction occurring between DTC and the dyes. Congo red and related compounds have been found to prevent the dialysis of DTC through a cellophane membrane. No such effect was noted with BE and HBE. Congo red and other sulfonic acid compounds form a precipitate with DTC but not with BE and HBE. The DTC-congo red precipitate is soluble in excess congo red but not in excess DTC.

Methods for the evaluation of compounds with curare-like action. K. K. KIMURA (by invitation) and K. R. UHNER *Department of Pharmacology, University of Illinois College of Medicine, Chicago 12, Illinois*. Curare-like compounds produce, on intralymphatic injection in frogs, progressive incoordination of spontaneous movements, loss of righting reflexes, and flaccid paralysis. By comparing the effects of indirect and direct stimulation of the muscle, the site of action of the drug can be verified with ease. Effects on other tissues—especially on the circulation, may be observed. Reversibility of the paralysis is a further indication of the selectivity of the action of the particular compound. Reproducibility of the effects of paralyzing agents on the muscles of the neck of the rabbit ("head drop" method) depends on the speed of the intravenous injection and on the size of the animal. The effects are usually not recorded objectively. Marked relaxation of the abdominal muscles precedes that of the neck muscles. The narrow margin between the "head drop" dose and the fatal dose too often results in the loss of animals. Intravenous injection in mice, likewise, produces head drop. This method is more economical and allows the statistically valid determination of the "head drop" dose on a uniform population. By determining the LD<sub>50</sub> under identical conditions, the margin between paralyzing and lethal doses can be accurately assessed. Neither the rabbit nor the mouse "head drop" method establishes the site of action of a drug. Potential curare-like compounds are, therefore, submitted to tests on frogs, before their effects are further studied on mammals.

The effect of pitressin on the hemodynamic responses of epinephrine and N-isopropyl-nor-epinephrine. THEODORE O. KING *Department of Pharmacology, Georgetown University School of Medicine, Washington, D. C.* In a series of experiments on dogs, doses of epinephrine hydrochloride

(0.25 cc and 0.5 cc of a 1:100,000 solution) and of N-isopropyl-nor-epinephrine (0.25 cc of a 1:100,000 solution) were administered by vein before and after the administration of pitressin. The pressor components of both drugs were potentiated by pitressin (see Table I), both as to height and duration. The vasodepressor effect of N-isopropyl-nor-epinephrine (%1024) was usually abolished or

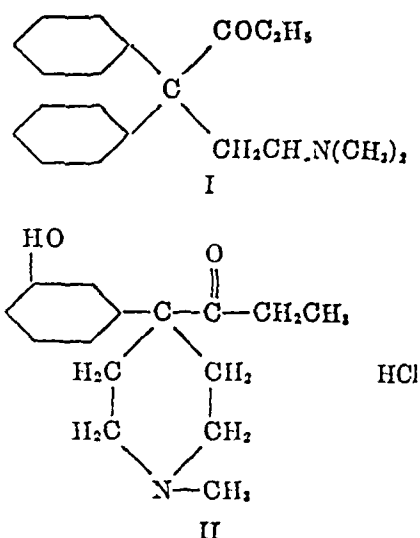
TABLE I

| Total doses                   | Control             | Pitressin (total)  |                    |                    |
|-------------------------------|---------------------|--------------------|--------------------|--------------------|
|                               |                     | 0.5 unit           | 1 unit             | 2 units            |
| Epinephrine HCl,<br>1:100,000 |                     |                    |                    |                    |
| 0.25 cc                       | +24 <sup>(11)</sup> | +32 <sup>(9)</sup> | +30 <sup>(7)</sup> | +36 <sup>(4)</sup> |
| 0.50 cc                       | +26 <sup>(8)</sup>  | +34 <sup>(8)</sup> | +37 <sup>(8)</sup> | —                  |
| %1024, 1:100,000              |                     |                    |                    |                    |
| 0.25 cc                       | -23 <sup>(10)</sup> | -2 <sup>(4)</sup>  | +1 <sup>( )</sup>  | +12 <sup>(1)</sup> |

Ave hemodynamic effects of epinephrine compounds before and after pitressin (nos in parentheses = number of experiments). Plus indicates pressor, minus sign depressor effects.

at least reduced by pitressin. When the pitressin effects wore off, the injection of additional pitressin again produced enhancement of the effects of the epinephrine compounds. In those experiments where pitressin failed to affect potentiation of epinephrine B.P. elevations or reversals or the N-isopropyl-nor-epinephrine vasodepression, administrations of 0.1 mg of ergotamine per Kg produced potentiation and reversal respectively. It is suggested that the administration of a non-specific vaso-constrictor by opposing the vasodilator component of the above drugs enhances the pressor action of these agents and that the potentiation of epinephrine by cocaine (?), ephedrine or ergotamine is in part due to a non-specific vaso constriction. Additional factors, of course, cannot be excluded. It may also be stated that in a statistically significant number of cases ergotamine reversed the depressor action of N-isopropyl-nor-epinephrine without accelerating the heart rate during the period of the actual rise of blood pressure.

**Further studies on synthetic analgesics.** A. C. KIRCHHOFF, *The Division of Anesthesiology, University of Oregon Medical School, Portland, Oregon.* Two of the drugs reported by the United States Technical Intelligence Team as synthesized by the I. G. Farbenindustrie in addition to methadon have been given a limited clinical trial at the University of Oregon Hospitals. One of these, called by the Germans %10582 (Formula I), was found to be rather inefficient in comparison with methadon. Analgesia was noted in 50 mgm doses but side actions were too frequent to continue the study. The other, %10720 (Formula II), called also Keto-Bemidone, in a very limited trial appeared to



merit further study since excellent analgesia was obtained with minimal side actions.

**Comparison of anesthetic action of acetanilid, tricaine (MS-222) and aliphatic depressants.** THEODORE KOPPAVIA and ALEXANDER G. KARCZMAR (by invitation), *Dept. of Pharmacology and Materia Medica, Georgetown University School of Medicine.* The kinetics of action of depressants depends, in *Amblystoma punctatum* larvae, on the anesthetic and on the larval stage. As to the latter, acetanilid (Fed. Proc., 6, p. 51), is a notable exception. Its speed of induction of anesthesia (see above, this issue) and its minimal effective concentration (1:1,500) are independent of the larval stage. Acetanilid action is reversible, the onset of action taking place within 5 to 15 minutes, and the recovery—from 2 to 3 minutes. Larvae, anesthetized with acetanilid, were subjected to multiple convulsive doses of metrazol, strychnine and prostigmine (o.c.). Convulsions were absent, but denarcotization failed to occur. Acetanilid also stopped convulsions already in evidence. Sub-anesthetic concentrations of acetanilid acted additively with subanesthetic concentrations of MS-222, nembutal and chloretone. Experiments in rabbits and dogs also indicate the CNS depressant action of acetanilid which is antagonized by metrazol; conversely, acetanilid prevents metrazol convulsions. Chloretone, tricaine, alcohol, and paraldehyde share with acetanilid the property of rapid reversibility of their action. On the other hand, the recovery time from chloral hydrate, nembutal and sodium barbital anesthesia is more prolonged. The ratio of recovery times for nembutal (1 hour) and for sodium barbital (4 hours) is comparable to that obtained in mammals. Experimental evidence shows that acetanilid is a general anesthetic requiring toxic doses for mammals but, for urodele larvae, concentrations below those of many aliphatic narcotics.

**Anesthesia with Cyclobutane** JOHN C KRANTZ, JR and C JELLEFF CARR *Department of Pharmacology, Univ of Maryland, School of Medicine, Baltimore* The anesthetic properties of cyclobutane were studied in dogs and monkeys. The anesthetic syndrome produced by cyclobutane is comparable to that of cyclopropane. However, cyclobutane appears to be the more potent of the two gases. Blood pressure remains essentially unchanged, and the form and rhythm of the electrocardiogram remain normal under cyclobutane anesthesia. The automatic tissue of the heart is sensitized to epinephrine when dogs and monkeys are anesthetized with cyclobutane. The oil/water coefficient of cyclobutane is higher than that of cyclopropane, which correlates with the higher anesthetic potency.

**Action of cysteine and of dimercaptopropanol in heart failure caused by sodium bismuth tartrate** OTTO KRAIER and ALFRED FARAH *Department of Pharmacology, Harvard Medical School* In the isolated heart of the dog (heart lung preparation) sodium bismuth tartrate causes impairment of contractility and disturbance of rhythm. Hearts, in apparently similar condition of work capacity (as revealed by competence test) prior to the administration of the bismuth compound, vary greatly in sensitivity. Some hearts, especially those which have been exposed to other substances and have been in the experiment for sometime, may require as little as 25 mg of sodium bismuth tartrate to produce severe failure, while in other hearts, especially in those freshly prepared, doses up to several grams may cause only moderate degrees of incompetence. Under certain conditions cysteine hydrochloride and dimercaptopropanol are able to restore normal function of the heart. The effect of cysteine is transient and the duration of action is proportional to the dose. If the first dose of the thiol compound is small in relation to the dose of sodium bismuth tartrate required to cause failure, no improvement occurs, rather, contractility is further impaired, or the heart is thrown into a state of severe arrhythmia or sudden stoppage of the heart is precipitated.

**Vasomotor activity of NU-1683 and other drugs upon the rat meso-appendix** DONALD C KUNZE (by invitation), J RICHARD R BOBB (by invitation) and HAROLD D GREEN *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina* Various drugs have been tested on rats using essentially the technique of the Zweifach and Chambers rat meso appendix test. The substances were tested as follows. Epinephrine was applied to the exposed mesentery in such dilution as to give a minimal constrictor response in the small muscular vessels. The drug to

be tested was then injected intravenously. The reactivity to the topical epinephrine was again noted. When the test drug caused a higher concentration of epinephrine to be required, the drug was designated as a dilator, and when a lower concentration of epinephrine was required, the drug was considered to be constrictor. Epinephrine (0.3-0.7 microgram/100 gm) had a constrictor action of slight degree (2X) for a short time (15 min). Ephedrine (0.1 mgm/100 gm) had a long lasting (40 min) constrictor action of small degree (2X). NU-1683(1 (m hydroxyphenyl) -N<sup>2</sup>-methyl ethylene diamine dihydrochloride, an experimental sympathomimetic compound), (Hoffmann-La Roche, Inc) (0.1 mgm-100 gm) showed a constrictor action greater in degree than ephedrine (8X) but of the same duration. With this drug, the largest venules in the field became congested and occasionally the flow of blood completely stopped, to begin again after a short period of time. The entire bed showed a visible slowing of flow, even without the application of epinephrine, most marked on the venous side. Etamon (tetraethylammonium chloride) (2 mgm/100 gm) showed a slight (2X), transient (10 min) dilator action. Priscol (2 Benzyl 4,5 Imidazoline) (0.1 mgm/100 gm) showed a dilator action of considerable strength (20X) for about 25 minutes.

The penetration of lead through the skin of the rat. FRIEDA M KUNZE (by invitation) and EDWIN P LAUG *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D C* In the past experiments designed to demonstrate whether lead penetrates the skin have been equivocal. They were based chiefly on the attempt to show significant elevation of urinary lead following cutaneous exposure, but suffered either from lack of sensitivity or to technical difficulties in restricting lead intake to the cutaneous route. A new method (Laug, et al, J Pharmacol and Exper Therap, 89 42, 1947) developed for the study of cutaneous penetration of mercury was applied to lead. This method utilizes the storage of lead in the kidney as a measure of the percutaneous penetration of lead. Rats were exposed to lead acetate, lead oleate, lead arsenate and lead tetraethyl either as aqueous or ointment preparations. From 77 to 150 mg (on the basis of metallic lead) were injected into a 29 (cm)<sup>2</sup> area of the skin of the back (8% of total body surface). The rats were wrapped in a special celluloid cuff which prevented oral contact. After 24 to 48 hours' exposure, the animals were sacrificed and the kidneys analyzed for lead. Small but significant penetration of lead followed exposure to the acetate, oleate and arsenate. Injury to the skin approximately tripled penetration. Markedly greater penetration occurred with lead tetraethyl,

the animals frequently dying within 24 hours from a total dose of 0.1 ml

**The daily dose of the mercurial diuretic and the maintenance dose to control congestive failure**  
 NATHANIEL T. KWIT (by invitation), WALTER MODELL, LAWRENCE W. HANLON (by invitation), JOSEPH G. BENTON (by invitation), ELAINE W. COTLOVE (by invitation), MORRIS PEARLMUTTER (by invitation), SIDNEY M. GREINBERG (by invitation), MILTON L. KRAMER (by invitation), and HARRY GOLD. In the current methods for the treatment of congestive failure, the organic mercurial diuretics are used at infrequent intervals, rarely shorter than 3 or 4 days. Indications that mercurhydrin is excreted in less than 24 hours led us to its daily administration in a regimen of treatment of patients with congestive failure. The regimen consists of a diet containing 1 to 1.5 gm of salt, 2 quarts of water, 12 mg digitoxin at one time, followed by 0.2 mg daily, and a daily intramuscular injection of 1 or 2 cc of mercurhydrin. The results obtained with this regimen in 110 hospital admissions with advanced congestive failure were compared with those in 502 similar admissions in 4 large hospitals, treated by the methods in current use, in which, among other differences, the mercurial is given at infrequent intervals. The comparison showed that with the daily dose of the mercurial, congestive failure subsided completely in 90 instead of 50% of cases in an average period of 6 days instead of 15 days, the mortality being 9 in place of 23%. A chart of the daily weight shows when the patient attains the "cure" or "dry weight" (in 6 days). The "dry weight" is maintained by prolonging intervals in the maintenance plan, to prevent recurrences of failure, which constitute about 18% of hospital admissions for congestive failure by the current methods of treatment.

**Pharmacological properties of trimeton, a new antihistaminic compound**  
 ANNETTE LABELLE and RICHARD TISLOW (introduced by O. Krayser). *Biological Research Laboratories, Schering Corporation, Bloomfield, New Jersey*. Trimeton ( $\gamma$ -Phenyl -  $\gamma$  - (2 - pyridyl) - N, N - dimethylpropylamine) prepared by Drs. Speiber, Papa and Schwenk in the Chemical Research Division was studied for its potency, toxicity and other pharmacological properties. It prevented the lethal effect in guinea pigs of 1.1 mgm/kg intravenous histamine dihydrochloride. The effective dose 50 per os was approximately 2 mgm/kg, and subcutaneously 0.1 mgm/kg. The protection afforded by 51 mgm/kg orally lasted from 8 to 16 hours. 110 mgm/kg orally, or 1.6 mgm/kg subcutaneously protected guinea pigs for over 2 hours in a histamine aerosol mist in which the control animals died within 5 minutes. 24 mgm/kg per os protected guinea pigs sensitized with species-

different sera from anaphylactic death. Acute toxicity tests performed in mice, rats, guinea pigs and dogs by various routes compared favorably with other antihistaminic drugs in use. Examination of the blood and the organs of immature rats fed 25 mgm/kg daily for 1 week did not reveal any difference between treated and control animals. In blood pressure experiments in dogs, Trimeton inhibited the depressor effect of histamine and potentiated the pressor effect of epinephrine. It had less spasmolytic activity than diphenhydramine hydrochloride as determined by the concentration effective in vitro in relaxing rabbits' intestine contracted with BaCl<sub>2</sub> or doryl. Trimeton did not have any antipyretic action as measured by a modification of the Barbour method. Solutions of Trimeton were less irritating to the rabbit eye and less anaesthetic to the guinea pig cornea than diphenhydramine hydrochloride.

**Preliminary studies in the toxicity of beryllium**  
 the effect of intratracheal injections in experimental animals. CHARLES W. LABELLE (by invitation) and MARTHA REID CUCCI (by invitation) (Introduced by Harvey B. Haag). With beryllium and its compounds are introduced into the lungs of rats via the trachea, the MLD for metallic dust and the insoluble compounds—oxide, carbonate, and carbide—are all greater than 200 mg of material per kilogram of body weight. The MLD of the soluble compounds, beryllium fluoride, sulfate, and oxyfluoride, was 15, 10, and 2 mg of salt per kilogram of body weight. Evidence is presented tending to show that the acute deaths result from a direct interference with lung function rather than a specific toxic effect of beryllium. The insoluble materials, while failing to show any distinct toxic effects of an acute nature, nevertheless lead to a more chronic type of damage resulting in a high rate of mortality after 100 to 250 days. Death is preceded by a series of inflammatory and necrotic changes in the lung, while changes in growth rate and increases in the polymorphonuclear leukocytes of the blood seem to offer the greatest aid in judging the progress of the reaction in intact animals. The pathological picture at death is not identical with that seen in man, and a series of concomitant exposures to debilitating agents such as mold infection, heat, and cold, has failed to alter the character or progress of the response.

**Analysis of dust and fume hazards in a beryllium plant**  
 SIDNEY LASKIN (by invitation), ROBERT A. N. TURNER (by invitation), and HERBERT E. STOKINGER (by invitation) (Introduced by Harvey B. Haag). A survey has been made of the industrial health conditions of a plant engaged in the manufacture of beryllium metal and alloys. The survey included an analysis of the dust and fume concentrations of the plant and a correlation

of these results with a past medical history of beryllium poisoning. The medical history of the plant from 1943 to 1946 showed 136 cases of toxicity attributed to beryllium compounds. Cases, for the most part, were confined to the preparation of the sulfate, the fluoride, the metal and the beryllium copper alloy. Typical symptoms are described. Dermatitis and skin ulcers accounted for 50% of all cases. Among the respiratory manifestations, chemical pneumonitis represented the severest form of the toxic symptoms, several deaths were reported in 1943. The general history showed a shift in case frequency away from the sulfate and beryllium alloy processes following improved working conditions at these sites. At the beryllium fluoride and metal areas, frequencies increased, reaching the greatest proportions in 1946. The safety index ranged from 63.5 to 238.1 per million man hours of exposure. Acceptable normal plant practice is 4. The amount of atmospheric contaminants in selected areas was sampled for concentration and particle size, the selection being based on past medical history of hazard or on obvious contamination at the time of the survey. Sampling equipment consisted of a Modified Cascade Impactor, Filter Paper Dust Sampler, and a MSA Midget Impinger. Near the fluoride furnace, fluoride concentrations—1000 fold that of beryllium—suggested a fluoride hazard in itself. Beryllium concentrations during operation averaged 0.065 mg/m<sup>3</sup> with a particle size of 2.46 microns (mass median). During operation of the beryllium metal furnace, beryllium concentrations varied from 1.4 to 4.7 mg/m<sup>3</sup>. Fluoride samples were in the order of 1.7 mg/m<sup>3</sup> and the mass median particle size averaged 0.86  $\mu$ .

Near the rotary kiln drier the beryllium dust concentrations varied from 0.050 to 0.53 mg/m<sup>3</sup>. Fluoride concentrations were of the same order of magnitude. The particle sizes, however, were quite large, ranging up to mass medians of 10 micra.

Several beryllium ores contained radioactive impurities. One lot was found to contain uranium concentrations up to 4% and thorium to 0.4%. The question of complicity of radioactive materials in beryllium toxicity is raised.

**Tissue distribution of a toxicant following oral ingestion of benzene hexachloride by rats.** EDWIN P. LAUG, *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.* A biological assay method (Laug, *J. Pharmacol. and Exper. Therap.* 86: 324, 1946) using the toxic response of the house fly has been applied to the determination of a toxicant in the tissues of rats exposed to gamma isomer of benzene hexachloride. Tests with the other isomers of benzene hexachloride, as well as the trichloro, heptachloro and octachloro derivatives, have shown that these compounds are only 1/100

as toxic as the gamma isomer. The latter is so toxic that 2 micrograms will kill 90 out of 100 flies within 24 hours. The toxic response to the gamma isomer is characteristic, when the insects are exposed to tissue extracts from animals poisoned with the gamma isomer their reaction is indistinguishable from that caused by the pure isomer. Therefore, it may be tentatively assumed that the toxicant in the tissues is the gamma isomer. Analyses of blood, liver, spleen, adrenal, muscle, brain, kidney and fat from rats which consumed diets containing 500 p.p.m. of the gamma isomer showed the presence of toxicant, the largest quantities (100 to 200 p.p.m.) occurring in kidney and fat. After one month feeding with benzene hexachloride the rats showed as much toxicant in their tissues as after four months. Detectable storage in liver, kidney and fat occurred even on diets containing 20 p.p.m. On the 500 p.p.m. diet approximately 1% of the injected benzene hexachloride appears as the toxicant in the urine, and only traces in the feces.

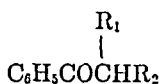
**Molecular architecture of cells.** C. D. LEAKE, *Univ. of Texas Medical Branch, Galveston.* Clark emphasized over a decade ago that the fundamental units in pharmacological activity are molecules of drugs and cells of living things. Drug action, however, is at a molecular level, and involves relations between molecules of the drug and molecules of the cell receptors. Thus, investigation of the molecular architecture of cells, including the organization of the various cell interfaces, becomes prerequisite to further clarification of the mechanism of drug action. Such study reveals the significance of short range (Van der Waals) polar association forces in drug receptor relationships. The chief chemical factors involved are hydrocarbon, polar, and ionic groups, with spacing and stereochemical factors determining the resulting forces of association. These forces vary in geometrical and inverse proportion to the distances between relevant groups, suggesting the sensitiveness of the association forces to the orientation of the molecules concerned. Slight changes in spacing, dipole charge, or ionization may for or split an association complex, thus accounting for high specificity and rapid enzyme reversibility. Competition between related chemicals in regard to receptor complexes in cell interfaces or other molecularly organized cellular structures results in mass law equilibria. The approach to pharmacological problems through a study of molecular architecture of cells and receptor complexes permits an attempt to apply quantum theory to pharmacological mechanisms, as has already been shown to be so promising in connection with various mutafacient agents.

**Studies on the relationship between chemical constitution and analgesic activity.** ROBERT A. LEHMAN, HERBERT S. KUPPERMAN (by invitation)

and JOY PHILLIPS (by invitation) *Department of Therapeutics, New York University College of Medicine, New York* A program for testing synthetic organic compounds for analgesic activity has been set up using a modification of the rat tail method (See, Hardy, Wolff and Goodell, *J Clin Invest*, **19**, 649 (1940) and D'Amour and Smith, *J Pharmacol*, **72**, 74 (1941)) The present technique consists of stimulation of the tip of the tail of the rat with a light beam of constant intensity and duration It will be shown that under these conditions a linear relationship exists between the logarithm of the dose and the probit of the percentage of a group of animals failing to respond to the stimulus The procedure used in rapidly screening a large number of compounds will be described in detail and results will be given for the several series listed below From these data an attempt will be made to point out those structural features which appear to contribute to or are associated with analgesic activity



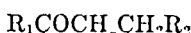
Where R is a short chain acyclic group This series has been found to possess little or no activity



Where R<sub>1</sub> and R<sub>2</sub> are usually heterocyclic groups



Where R<sub>1</sub> is usually an aryl, R<sub>2</sub> short chain alkyl and R<sub>3</sub> a heterocyclic group



Where R<sub>1</sub> is aryl or substituted aryl and R<sub>2</sub> is usually a heterocyclic group



Where R<sub>1</sub> and R<sub>2</sub> include hydrogen, alkyl, aryl, aralkyl and heterocyclic groups This series has been conspicuous in that marked activity is shown by many of its members

**Low toxicity and high therapeutic efficacy of combined sulfonamides** DAVID LEHR *Dept of Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City* Several sulfonamides, even if closely related, could be dissolved simultaneously to the full extent of their separate saturation levels without the occurrence of precipitation In the experimental animal the toxicity of the combinations sulfathiazole (ST)-sulfadiazine (SD), SD-sulfamerazine (SMD), SD-sulfapyridine (SP), SD-ST SMD, SD ST SP,

SD SMD sulfapyrazine, and SD-SMD sulfacetamide, was found to be strikingly low as contrasted to the high mortality from comparable dosages of any one of the N<sub>1</sub>-heterocyclic derivatives (D Lehr, *Brit Med J*, in press) Combinations of three sulfonamides were significantly less toxic than combinations of two Evidence derived from post mortem examinations and from chemical analyses of the blood, kidneys, and urine, proved that the low toxicity of sulfonamide combinations was due to the absence of crystalline deposition in the urinary tract (D Lehr, *Proc Soc Exper Biol & Med* **61** 393, 1917) The antibacterial effect of combined sulfonamides, tested in vitro, was essentially additive In some instances, combinations proved distinctly more active than one or several of their individual components at equal concentrations More than 900 unselected patients of all age groups, with acute systemic infections were treated with equal partial amounts of SD and ST or SD and SMD using the routine oral dosage In infants and small children the latter combination was injected also subcutaneously Therapeutic results were uniformly satisfactory and conspicuous in many instances because of the speed of improvement or cure Effective blood levels of sulfonamide and high drug concentrations in the urine were readily maintained Despite the intentional omission of alkalinization, crystalluria was infrequent and no signs of renal irritation were encountered Clinical studies with mixtures of three sulfonamides, as yet incomplete, indicate that the danger of concrement formation in the urinary tract can be entirely eliminated by combination therapy Hence, mixtures of partial dosages should replace the use of single sulfonamides in systemic therapy, since mixtures combine a high bacteriostatic activity with a minimal renal toxicity

**Studies on the toxicity and pharmacology of some synthetic antispasmodics** DAVID LEHR *Dept of Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City* The oral toxicity of di-(tributylethylamine) β-naphthol-3,6 disulfonate (S<sub>1</sub>), tributylacetamide (S), di-(tributylcarbinamine) β-naphthol 3,6 disulfonate (S<sub>6</sub>), tributyl ethyl nicotamide (S<sub>51</sub>), β diethylaminoethyl-N-tributylcarbamate (S<sub>50</sub>) and its hydrochloride (S<sub>50</sub>), was studied in albino rats, partly also in mice and rabbits The pharmacological action of these compounds with particular reference to antispasmodic activity was evaluated on the gut (rat, rabbit), on the uterus (rat, cat), on the rabbit eye, and the frog heart The vascular response was tested by perfusion of the frog hind legs and the isolated rabbit ear, as well as by the effect on the blood pressure of intact cats and rabbits Comparison was made with papaverine and atropine as standards for musculotropic and neurotropic spasmolysis, and in addition with



syntropan, trasentin, and profenil  $S_{80}$  was found to be the most toxic compound, followed by  $S_{10}$  and  $S_4$  which were about half as toxic, and  $S_1$  which had one third the acute toxicity of  $S_{80}$ .  $S_2$  and  $S_{31}$  were of such low toxicity that despite excessive dosages (15 and 10 gm/kg), a MLD could not be obtained. The pharmacological evaluation proved that all "S" compounds were essentially muscurotropic antispasmodics. In the intensity of this effect  $S_{31}$  equalled papaverine,  $S_2$  was twice as strong,  $S_1$  and  $S_4$  four times, and finally  $S_{80}$  and  $S_{80}$  about 5 times as strong. The latter two compounds produced irreversible changes on the isolated gut and frog heart.  $S_{31}$  caused slight stimulation of the frog heart and potentiation of acetylcholine action, whereas all other compounds induced only depression.  $S_1$  and  $S_{31}$  effected a brief blood pressure rise and stimulation of respiration. The other 4 compounds lowered the blood pressure. None of these drugs had any action on the iris by local application, but all caused irritation of the conjunctiva which was least marked from  $S_{31}$ .

**Influence of amidone, morphine and strychnine on the Straub-tail-test of white mice.** ALFRED LEINDORFER, *Department of Pharmacology, University of Illinois, College of Medicine, Chicago*. After the intraperitoneal injection of 0.1 cc of amidone HCl (1% solution), but regularly after the injection of 0.2 cc, the tail was characteristically elevated. This phenomenon was more quickly produced and more pronounced by soft stroking along the spinal column in the thoracic region after the injection. Similar responses were observed after the subcutaneous injection of 1 mgm morphine sulfate or after the subcutaneous injection of 0.15 cc strychnine sulfate (1:1000). In further experiments it was found that strong pressure applied to the lumbo-dorsal region of the spinal column might elicit elevation of the normal mouse tail without any injection. To elucidate further the site of origin, the spinal cord was dissected in the mid-thoracic region under light ether anesthesia, several hours later, amidone HCl, morphine sulfate and strychnine sulfate in the previous doses were injected, using the same route of administration. Whereas amidone HCl and morphine sulfate did not induce any tail reaction under these conditions, the injection of strychnine sulfate still elicited elevation of the tail, sometimes in connection with general tetanic convulsions, sometimes only the elevation of the tail took place. Further, after decerebration of mice, general decerebrate rigidity with convulsions and elevation of the tail were seen similar to that after amidone-HCl, morphine sulfate or strychnine injections. The elevation of the tail of white mice seems to be a normal reflex phenomenon, perhaps originated in the spinal cord. The development of this reaction seems to be facilitated either by increased activity

along the cerebrospinal pathways (due to amidone or morphine) or by increased excitability of the spinal cord (due to strychnine).

**The use of natural and synthetic fat emulsions for intravenous feeding.** HARRY H. LEVEEN (introduced by FRANK CO. TUI), *Department of Surgery, New York University College of Medicine*. A safe, metabolizable solution of fat for intravenous use would be significantly valuable in clinical nutrition. In preparing emulsions for intravenous use, extremely small particle sizes are essential to prevent fat embolization and to stabilize the emulsions. Since it is impossible to make emulsions of natural fats with satisfactorily small particle size by mechanical methods, emulsifying agents must be added to lower the surface tension between the fatty and the aqueous phases. However, fat films are an integral part of the cell membrane and since the emulsifying agents tend to make the fat film soluble, they disrupt cell and capillary membranes. Even non-toxic emulsifying agents, used heretofore, produce hemolysis of red cells and increased capillary permeability. The latter is especially noticeable as pulmonary edema. Death ensues from the sudden development of a shock-like state characterized by a rapidly falling blood pressure. For these reasons, it would seem impossible to prepare satisfactory emulsions of natural fats. Synthetic fats will form stable emulsions of extremely small particle size without emulsifying agents. The synthetic fats under investigation by the author include the esters of the higher fatty acids with sugars and polyhydric alcohols, the very short chain fatty acids and their esters, and the dicarboxylic fatty acids. The intravenous use of some of these synthetic fats is discussed.

**The physiological disposition of procaine in man.** PHILIP A. LIEF (by invitation), RAYMOND POET (by invitation) and BERNARD B. BRODIE, *Departments of Anesthesiology and Biochemistry, New York University College of Medicine, and New York University Research Service, Goldwater Memorial Hospital, N. Y.* Procaine has recently been administered intravenously to relieve pain and to remedy spontaneous cardiac arrhythmias caused by cyclopropane. The fate of Procaine in man was studied for information concerning the mechanism of these actions.

Procaine hydrochloride was given intravenously to various subjects. Procaine, diethylaminoethanol and p-aminobenzoic acid were identified in the urine. These substances were then estimated in urine and plasma. Urinary excretion accounts for about one per cent of administered procaine. Therefore, the largest fraction of procaine undergoes metabolic alteration. P-aminobenzoic acid (and derivatives) excreted is equivalent to about 80 per cent of procaine. Diethylaminoethanol excreted is equivalent to about 25 per cent of

administered procaine When diethylaminoethanol is administered intravenously only about 25 per cent is recovered in the urine The nature of the metabolic alteration of diethylaminoethanol has not been established Procaine disappears rapidly from plasma, only traces being found even during injection Appreciable concentrations of diethylaminoethanol and p aminobenzoic acid are found These results indicate that procaine rapidly hydrolyzes *in vivo* to p aminobenzoic acid and diethylaminoethanol Procaine also hydrolyzes rapidly in human plasma *in vitro* The rapidity with which procaine disappears from the body and the relative persistence of diethylaminoethanol suggest the possibility that the latter may be the active agent Preliminary experiments show that diethylaminoethanol is much less toxic than Procaine and may be administered orally in relatively large doses It is being tested for its analgesic properties and for its effect on the heart arrhythmias of cyclopropane anesthesia

**Absorption of salicylates from intestinal loops in the dog** E WILLIAM LIGON, JR (by invitation), ROBERT A MADDEN (by invitation) PAUL L DAVIS (by invitation) and PAUL K SMITH From the Department of Pharmacology, The George Washington University School of Medicine, Washington, D C It has been reported that sodium bicarbonate increases the rate of absorption of concurrently ingested salicylates In this study of salicylate absorption, to eliminate the hastening effect of alkali on gastric evacuation and more accurately control the pH of the gut contents, trained unanesthetized Thiry-Vella jejunal loop dogs were employed Solutions containing 200 mgm per cent salicylic acid were made isotonic by adding sodium chloride and/or sodium bicarbonate (pH 2.5 to 7.8) and passed through the gut loops at about 300 cc per hour Plasma and urine content of salicylate were determined spectrophotometrically at two hours Fifty one experiments on three dogs showed better correlation of plasma level with the pH of the outflowing perfusate than with the lower pH of the inflowing solution Maximum plasma levels of approximately 10 mgm per cent were obtained at outflow pH 5.0 to 5.8, with values decreasing to approximately 4 mgm per cent at either extreme Urine content of salicylate was an insignificant correction factor in effect of pH on rate of absorption It has been shown that acetylsalicylic acid injected intravenously in the dog is rapidly hydrolyzed Experiments were designed to demonstrate whether or not acetylsalicylic acid is hydrolyzed prior to absorption, using aspirin solutions in the same dogs in a similar manner No greater concentration of free salicylate was found in this perfusate (pH 5.4) than in control solutions, suggesting that aspirin is hydrolyzed after absorption

**The toxicity of some surface active agents on the frog heart** ALFRED G LISI, (introduced by CHARLES M GRUBER) Department of Pharmacology, Jefferson Medical College, Philadelphia, Pa In an effort to determine the possible effect of residues left on glassware cleaned with the aid of the newer surface active agents, a study was made of the action of certain commercial preparations on the excised, perfused frog heart An all glass cannula was used which has a side arm for maintaining a constant level of solution The following preparations were employed sodium lauryl sulfate (Du Pont's "sodium 'lorol' sulfate PT"), a polymerized ethylene oxide condensation product (Igepal CA Extra, an aryl alkyl sulfonate (Nacconol NRSF) and a sorbitan monooleate polyoxyalkalene derivative (Tween 80) The most useful criterion was found to be the observation of a minimal decrease in the height of contraction as recorded on a kymograph Ringer's solution was perfused through the heart during control periods and between applications of the surface active agent Appropriately diluted solutions of the test substances in Ringer's solution were applied to the heart for six minute periods The inhibition regarded as "minimal" was such that it could be readily seen by comparison with the preceding and following control periods Concentrations of solution could only be recorded in terms of per cent since the materials tested are not pure compounds In general terms, the surface active agents tested were toxic in the range of 0.0005% - 0.005% solutions For comparison, potassium dichromate was tried and its comparable toxicity was around 0.01% - 0.02%

**A simplified method of evaluating dose-effect experiments** J T LITCHFIELD, JR and F WILCOXON (by invitation) Chemotherapy Division, Stanford Research Laboratories, American Cyanamid Company, Stamford, Connecticut By means of tables and nomographs to be presented, an approximate solution of dose effect curves can be made quickly and easily by the use of a slide rule and logarithmic probability paper No elaborate computation and no use of logarithms or probits is required The method includes a test of the goodness of fit of the line to the data by means of a  $(\text{Chi})^2$  test, as well as the estimate of the median effective dose ( $\text{ED}_{50}$ ) and its 19/20 fiducial limits The method makes possible also the estimation of the 19/20 fiducial limits of  $S$  Since  $S$  with its limits is a function of the slope constant ( $b$ ) and its standard error, the degree of parallelism between two assays can be tested If the curves of two assays are parallel within the limits of error, the ratio of potency may be computed and the 19/20 fiducial limits established

**Anticonvulsant actions of trimethadione-phenobarbital and trimethadione-diphenylhydantoin**

combinations S LOEWY *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah* (a) Protection against metrazol convulsions and (b) abolition of tonic hindleg extension phase of maximal electroshock seizures were measured in various species, by determining the protective doses ( $PD_{50}$ ) of the individual drugs and the isobols, as previously described, of the drug combinations. The anticonvulsants trimethadione (T), phenobarbital (Ph) and diphenylhydantoin (D) were injected intraperitoneally, metrazol was given subcutaneously (rats, mice) or intravenously (cats) (a) Whereas no antimetrazol synergism of T+Ph was demonstrable in mice and cats or of T+D in rats, T moderately synergized Ph in rats and D in mice, but only when the dose ratios T/Ph and T/D were low (b) *Antishock* synergism of marked degree was demonstrable in all cases studied (T+Ph in rats and cats, T+D in rats). However, the synergism of T+Ph in rats was limited to high T/Ph dose ratios, whereas antagonism prevailed in the range of small T/Ph ratios. T and D (single D dose, four hour interval) synergized mutually in all dose ratios, also repeated D doses were markedly synergized by as little as  $\frac{1}{3} PD_{50}$  of T. Since efficacies in petit and grand mal are correlated with antimetrazol and antishock activity, respectively, these results may be instructive for selecting optimal therapeutic combinations. They suggest that in petit mal a T regimen might not profit from small Ph or D additions, but vice versa, in grand mal, a T regimen might be improved by small Ph additions—but not vice versa—and T+D combinations in all ratios might offer advantages.

The response and the mechanism of the response of the duodenum to morphine TED A LOOMIS (introduced by JAMES M DILLE) *Department of Pharmacology, University of Washington School of Medicine, Seattle, Washington*. The muscular response of the duodenum to intravenously administered morphine was studied in intact anesthetized dogs. Apparatus designed to measure simultaneously the changes in circular and longitudinal muscle activity was used. The predominant effect of morphine on the circular muscle group was found to be an increase in its many phases of activity. The predominant although not completely consistent effect on the longitudinal muscle group was found to be a decrease in its many phases of activity. The type of response was found to be independent of the original state of tonus and of the dose over a range of 0.1 to 1.0 mgm per kg. In the presence of a barium spasm, morphine produced little or no alteration of muscle activity. In the presence of physostigmine or atropine the response to morphine was similar to the normal response produced by morphine. The data obtained indicates that the site of action of morphine is

distal to the acetylcholine choline esterase mechanism resulting in stimulation of the circular muscle group and depression of the longitudinal muscle group.

Comparative effects of sparteine and quinidine on isolated frog heart GO LU (Fellow, American Bureau for Medical Aid to China Inc., by invitation) *From the Department of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco 15, Calif*. Using a modified assembly of the Hartung Clark double cannula and the Straub ventricle methods sparteine and quinidine were compared on known characteristics of the isolated frog heart. The predominant effects of sparteine were purely depressant, qualitatively resembling quinidine in all essential respects, i.e., bradycardia, typical lengthening of the refractory period (also demonstrated on isolated skeletal muscle), relaxation of cardiac muscle, decreased contractility and cardiac output, abolition of extrasystoles, and prevention and arrest of auricular fibrillation. These changes were either abolished or prevented by atropinization, and therefore were muscular in origin. The apparent initial stimulation of sparteine indicated by moderate increases in amplitude of contractions and stroke and minute output (not produced by quinidine) is presumably due to the slowing in rate and relaxation of cardiac muscle. Ready conversion of this initial action to a marked depression, tendency to diastole, and lack of benefit of sparteine on failing hearts were further evidences supporting the slowing mechanism of "stimulation," simulated in all essential respects by cooling of the sinus node. The sparteine arrested heart was readily reversed by washing with Ringer's solution or addition of epinephrine, calcium, and caffeine, but not so readily quinidine arrested hearts. Toxic concentrations of sparteine were somewhat higher than those of quinidine. According to these results, sparteine satisfies the essential requirements of a quinidine like drug, with a somewhat lower toxicity, a freedom from cinchonism, and a higher solubility permitting possibly better absorption and easier adjustment of dosage. Sparteine seems worthy of further clinical trial.

Some pharmacological actions of isomers of methadon F P LUDUENA, LLOYD C MILLER, ESTELLE ANANENKO (by invitation) and J D FRICK (by invitation) *From Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N Y*. Using the radiant heat method of Ercoli and Lewis the analgesic potencies of *l*- and *d*-methadon, (6-dimethylamino-4,4-diphenyl-3-heptanone), and the *dl* form of a structural isomer, ("Win 1783", 6-dimethylamino-4,4-diphenyl-5-methyl-3-hexanone), were determined by subcutaneous injection in rats. The approximate threshold analgesic doses were 1, 20 to 30, and 4

mg/kg, respectively. Thus the *l*-isomer is responsible for most of the analgesic action of racemic methadon. This observation confirms the report of Thorp, Walton and Ofner on *l*- and *dl*-methadon.

Win 1783, which differs from methadon only in the position of the methyl group, is about half as active as *dl*-methadon, but the respective therapeutic indices are about the same, as judged from toxicity data described elsewhere in these Proceedings (Hoppe and Miller). On barbitalized dogs quantitative and qualitative differences were observed between the actions of *d*- and *l*-methadon given intravenously. The first injection of *l*-methadon lowered the blood pressure, subsequent injections produced a moderate rise after a fleeting fall. The *d*-isomer produced only a depressor effect. Other pharmacodynamic effects of these analgesics on anesthetized and unanesthetized dogs will be discussed.

The protective action of BAL on experimental poisoning by lead, tungsten, copper and Paris green. LEHMAN M. LUSKY (by invitation), HERBERT A. BRAUN and EDWIN P. LAUG. *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.* We previously reported that BAL enhanced the toxic effect of lead salts administered by intramuscular or intraperitoneal injections to rabbits. This led to investigations to determine whether the increased toxicity was due to the formation of a toxic complex or systemic mobilization. In the present studies single intravenous doses of lead acetate (20–40 mg/kg) were administered intravenously to young rabbits. Initial treatment of BAL (30 mg/kg) was followed by descending subcutaneous doses during the 30-day experiment. Comparison of the mortality curves of control and BAL-treated animals indicates that BAL reduced the toxicity of intravenously administered lead acetate by 50%. The bone of BAL-treated animals contained smaller concentrations of lead than the controls. Control animals given 110 mg/kg sodium tungstate intramuscularly died within 5 hours. BAL (30 mg/kg) intramuscularly in the opposite leg within 20 minutes after the tungstate injection saved all rabbits from this dose of sodium tungstate. The effect of BAL on experimental copper poisoning is striking. Fifteen control rabbits intravenously injected with cupric sulphate (20 mg/kg) died within 30 minutes. BAL (30 mg/kg) intravenously after the injection of cupric sulphate saved 14 of 15 rabbits. The antidotal effect of BAL against Paris Green was demonstrated in rabbits. Orally, 25 mg/kg was fatal to 15 of 18 animals, while 13 of 18 were saved with BAL started at 30 mg/kg twice a day and continued in descending doses for 8 days.

Influence of benadryl and pyribenzamine on the neuromuscular system of rats. DAVID I.

MACHT and THOMAS HOFFMASTER (by invitation). *Division of Pharmacology, Laboratories of Sinai Hospital, Baltimore*. Inasmuch as these two drugs produce occasional reactions particularly on the part of the Nervous System, studies were made on the behavior of white rats by methods described elsewhere (Macht D. I. *Experimental Medicine and Surgery*, 1913, Volume I, Page 260). Three sorts of experiments were performed: (1) on the behavior of rats in a circular maze giving data on neuromuscular activity and on discrimination or "choice" reactions; (2) on the coordination of rats running across a tightly stretched horizontal rope; (3) on neuromuscular work performed by rats climbing a vertical rope. Doses of both Benadryl and Pyribenzamine ranged from 0.1 mg to 2 mgs. Injections were made either intraperitoneally or subcutaneously. As little as 0.2 mgs of either drug produces a depression in activity within 15 minutes. Doses of 0.5 mg and more cause marked depression. When larger doses (0.5–2.0 mgs) are injected in rats weighing 150–200 gm, the maze performance is impaired more than the rope walking and climbing, thus pointing to the CNS being more affected than the muscular responses. Even after doses of 5 mgs the animals recovered in a few days.

Phytotoxic effects of normal, pathological, and irradiated blood sera. DAVID I. MACHT and MARCUS OSTRO (by invitation). *Departments of Pharmacology and Radiology, Sinai Hospital, Baltimore*. This being the 25th anniversary of the senior author's researches in Phytopharmacology, his experiences with thousands of blood specimens are reviewed and analyzed, and new discoveries are here presented. Blood sera of all higher animals, except reptiles, give normal indices of root growth (ranging 70% to 75%) for *Lupinus albus* seedlings. Reptilian sera are toxic. Sera of virus diseases are weaker than normal sera. The following conditions yield specific toxic indices, which are useful in diagnosis: Menstruation, Pernicious Anemia, Pemphigus, Leprosy and Trachoma. P.V. serum is phytotoxic, all other anemias and leukemias are not. It is detoxified in quartz by ultraviolet rays of a mercury vapor lamp. So are patients in vivo. Pemphigus serum is unaffected by ultraviolet, but detoxified by specially filtered very short  $\gamma$  rays. Such deep  $\gamma$ -rays over the liver and spleen produce favorable therapeutic effects and have saved several moribund cases (*Urological and Cutaneous Review*, November, 1947). Menotoxin, Trachoma and Leprosy sera are unaffected by roentgen rays. Numerous serum specimens from psychotic patients revealed that all psychoses whether organic or functional are phytotoxic, and are detoxified *in vitro* by special  $\gamma$  rays. Animals receiving penicillin affect root growth, and patients' blood levels can be estimated phyto-

pharmacologically "Vernalized" or "Jarovized" seedlings yield a specific reaction with Psoriasis blood not shown by normal plants (Edwin C Miller, *Plant Physiology*, Second Edition p 1091)

The distribution, excretion, and blood levels of antimony following administration of antimonials to various mammalian species THOMAS H MAREN and GILBERT F OTTO (introduced by E K MARSHALL, JR) The trivalent and pentavalent antimonials in current clinical and experimental use show wide differences in their distribution and excretion, there are also significant differences in the behavior of these drugs in various species of animals Compounds used included among others tartar emetic, monosodium antimony thioglycolate, "fuadin", and "solustibosan" Pharmacological data was obtained for the white mouse, hamster, cotton rat, guinea pig, rabbit, dog, and man In these species natural or experimental infection of several types have long been treated with antimonials, in the present study uninfected animals as well as those with filariasis and schistosomiasis have been used in an attempt to provide a pharmacological basis for therapy Following intraperitoneal or intravenous injection the trivalent compounds give rise to deposition of antimony principally in the thyroid gland and the liver Pentavalent compounds result in antimony concentration in the spleen, usually with lesser amounts in the liver Quantitative differences were observed with different species In dog and man, following trivalent antimony, there is approximately 5 times as much metal in red cells as in plasma After pentavalent compounds, on the other hand, plasma concentrations at the peak were 10 times that of the cells Excretion was extremely variable with the species In hamsters, mice, and white rats receiving trivalent antimony, the metal was eliminated mainly in feces In guinea pigs, rabbits, dogs, and in man it is excreted largely in urine In all species receiving the pentavalent drugs, the metal was rapidly eliminated in the urine

**Antagonism of nicotine and atropine** AMEDEO S MARRAZZI *Department of Pharmacology and Therapeutics, Wayne University College of Medicine, Detroit, Michigan* Marrazzi (J Pharmacol 65 18, 1939, Fed Proc 6 354, 1947) has shown that atropine is capable of blocking transmission in sympathetic ganglia Therefore, a distinction, such as a so-called nicotinic action as opposed to a muscarinic, based on experimental procedures dependent on an alleged immunity of ganglia and nicotine to atropine requires re examination The rise in blood pressure caused by nicotine is considered a characteristic action It seemed desirable, therefore, to determine whether it may be modified by the prior injection of atropine Such an effect on the circulatory system, which is controlled by

so many quite variable factors including compensatory reflexes is quite apt to be masked Accordingly, we found a constant pressor effect of nicotine and its depression by the prior intravenous injection of atropine in only the occasional experiment where conditions appeared to have fortuitously favored its detection However, on eliminating the vagi and the carotid sinuses more regular results are obtained showing a control rise on the intravenous injection of nicotine, the absence or reduction in the expected rise due to nicotine when it is injected after atropine and a subsequent control rise again with the nicotine alone The masking of the nicotine atropine antagonism on circulation and on other effector systems, the mechanism of the fall in blood pressure after intravenous atropine and the bearing of the nicotine atropine antagonism on concepts of cholinergic transmission will be discussed

**Synthetic curare compounds III d-N-methyl-chondrodendrine iodide and d-N-methyl-O-methyl-chondrodendrine iodide** DAVID FIELDING MARSH, CLARK K SLEETH (by invitation), and ELTON B TUCKER (by invitation) *Departments of Pharmacology and Medicine, West Virginia University School of Medicine, Morgantown, W Va* d Chondrodendrine was isolated from *Pareira brava* The curariform activity of its derivatives, d-N-methyl-chondrodendrine iodide ("beberine dimethiodide", CM), and d-N-methyl-O-methyl-chondrodendrine iodide ("beberine methylether methiodide", CMMMeO), was determined in comparison with the corresponding isomers, d-tubocurarine chloride pentahydrate (dT), and d-O-methyltubocurarine iodide trihydrate (dTMeO) The relatively high curariform

|  | dT Cl       | dTMeO I      | CM I       | CMMMeO I   |
|--|-------------|--------------|------------|------------|
| Albino rats<br>LD 50                                   | 0 27(0 22)* | 0 032(0 022) | 0 55(0 39) | 0 37(0 27) |
| Rabbits<br>Head drop 50                                | 0 12(0 10)  | 0 016(0 011) | 0 23(0 16) | 0 11(0 08) |
| Holiday head<br>drop                                   | 0 15(0 12)  | 0 020(0 014) | 0 27(0 19) | 0 13(0 09) |
| LD 50  | 0 35(0 28)  | 0 040(0 027) | 0 40(0 28) | 0 28(0 17) |
| Cat gastrocnemius<br>muscle<br>Equivalent<br>paralysis | 0 05(0 04)  | 0 007(0 005) | 0 40(0 28) | 0 03(0 04) |
| Man<br>Head drop                                       | 0 15(0 12)  | 0 030(0 020) | 0 60(0 43) | 0 15(0 11) |

\* All doses given in milligrams/kilogram body weight Dose of contained curariform ion given in parenthesis

activity, lack of undesirable side effects, and ready availability of these chondrodendrine derivatives make their clinical investigation seem reasonable

**Preliminary data on rat feeding with beryllium** ELLIOTT A MAYNARD (by invitation), WILLIAM L DOWNS (by invitation), and HAROLD C HODGE (by invitation) (introduced by HARVEY B HAAG At a dietary level of 5%, Be sulfate and Be carbonate completely inhibited growth of rats and caused some mortality. A rickets-like condition occurred in these rats. Food intake was reduced by about 50%, but this did not account for all growth inhibition as shown by a paired feeding experiment. Return to stock diet after a prolonged period of ingestion of Be salt allowed rapid but not complete growth recovery, and healing of the rickets occurred in a few days. In turn this was followed by the appearance of a radio-translucent area extending into the shaft of the bone from the metaphyseal space. Be sulfate was more toxic to old rats than to weanlings, but with Be carbonate mature rats seemed to be more resistant than weanlings. Be carbonate at a level of 2.3% of the diet caused some weight depression in rats. Be metal at a 10% dietary level and Be oxide at a 5% dietary level did not affect the growth of the rats.

**Some aspects of the action of curare on the central nervous system** E L McCawley *From the Laboratories of Pharmacology and Toxicology and the Laboratory of Neuroanatomy, Yale University School of Medicine, New Haven, Connecticut* Cats were used in the majority of experiments reported here, although dogs and rabbits were also employed. Records were made of the cortical electroencephalogram, cerebrospinal fluid pressure, the electrocardiogram and blood pressure. In all cases artificial respiration, 23 strokes per minute, was supplied through a tracheal cannula or endotracheal catheter. Curare, "Intocostin" and d-tubocurarine chloride, and tetraethyl ammonium bromide were used. Since the effect of these drugs is transitory a constant rate of injection was employed such that the rate of administration exceeded the rate of elimination or destruction. By varying this rate of injection it was found that the appearance of symptoms and events followed a parabolic function of time and total dosage. In the unanesthetized animal doses of curare less than those required to produce paralysis of the intercostal muscles produced clonic convulsions. The EEG was characterized by high frequency spikes of considerable voltage, i.e., similar to that of grand mal. Tetraethyl ammonium elicited the same effect, as did also very small doses of curare placed in the cisterna magna. This phenomenon was prevented by the depressant drugs pentobarbital and thiopental and also by morphine. In the anesthetized animal cortical depression from curare appeared at 200-300 units/kg body weight and the rate of administration exceeded 0.5 cc/minute. Cortical brain waves were depressed for brief peri-

ods of time and finally disappeared entirely (iso electricity). However, although the cortical brain waves were eliminated, trains of spikes 11-18/sec and, characteristic of thalamic activity (or neighboring structures) appeared. Animals whose cortical activity was depressed by curare could not be revived. Although in certain animals the heart was kept beating by artificial respiration for 20 hours and the drug eliminated, there was no return of brain function. Physostigmine, neostigmine, guanidine, peripheral anticholinergic agents, had no demonstrable central effect. "Metrazol" alone produced a temporary return of cortical activity.

**The action of p-aminosalicylic acid (PAS) in experimental tuberculosis** W T McClosky, M I SMITH and J E G FRIAS (by invitation) *Division of Physiology, National Institute of Health, Bethesda, Maryland* The acute and chronic toxicity and fate of p-aminosalicylic acid (PAS) were studied in rats, rabbits and guinea pigs and its effectiveness in experimental rabbit and guinea pig tuberculosis ascertained. The acute toxicity of PAS is low. Rats tolerated 20 gm/kg when injected intravenously, and in guinea pigs on oral administration the approximate LD<sub>50</sub> was 30 gm/kg. Continued oral administration to guinea pigs in daily doses of 0.5 gm/kg showed cumulative action since 50% of the animals died after 18 doses. The compound is well absorbed from the gastrointestinal tract, is retained for 3 to 6 hours, and is nearly completely excreted in the urine within 24 hours, for the most part in the conjugated form, probably as the acetylated product. In rabbits infected with 0.1 mg bovine strain (Ravenel) tubercle bacilli i.v. and treated with PAS in doses of 0.2 to 0.5 gm/kg daily the average extent of tuberculous involvement for a group of six animals was 3.0 as compared with 5.1 for the untreated controls, whilst a similar group treated with streptomycin, 40 mg/kg/day, showed no lesions in five of the animals and minimal lesions in one. A group of animals treated with both drugs had no evidence of tuberculosis. Four groups of guinea pigs, ten each, inoculated intraperitoneally with 0.5 mg tubercle bacilli human strain A27 and treated with PAS 0.3 to 0.5 gm/kg/day alone or in combination with 20.0 mg/kg/day of streptomycin showed an average extent of tuberculous involvement as follows: Untreated controls 10.7, treated with PAS 7.4, treated with streptomycin 1.5, treated with both drugs 1.3. Thus the chemotherapeutic effectiveness (ratio of T.B. in controls/treated) of PAS was 1.4, of streptomycin 7.1 and of combined therapy 8.2, which is no greater than the sum of effects from the individual components. It is concluded that the chemotherapeutic efficacy of PAS in tuberculosis is slight compared with the sulfones, and unlike the sulfones it gives no indication of potentiation with streptomycin.

Acute vapor toxicity to mice and local irritant effects in the rabbit of methyl pentadiene W A McOWIE (introduced by HAMILTON H ANDERSON) *Division of Pharmacology and Experimental Therapeutics, University of California Medical School and College of Pharmacy, San Francisco, California* The material used was a mixture of approximately 85% of 2 methyl-1,3 pentadiene and 15% of 4 methyl-1,3 pentadiene. Stock white mice were exposed in a chamber to the vapors of this low boiling (75°C) olefinic hydrocarbon by diluting nitrogen and vapor mixture with different rates of flow of air, so as to obtain a dosage mortality relationship (see table). The periods of exposure varied from 30-270 minutes and the concentration of vapor from 3-150 mgm /l

Dosage mortality relationship\* upon exposure of mice to vapors of methyl pentadiene

| ct product (mgm /l X minutes) | No. of mice dying within 96 hours/Total no. mice used |
|-------------------------------|---|
| 3750                          | 0/6   |
| 5400                          | 2/6   |
| 12000                         | 8/12  |
| 18400                         | 4/6   |

\*  $L(ct)_{50}$  = Product of concentration X exposure period causing 50% mortality

An  $L(ct)_{50}$  of 10,000 mgm min /l was estimated from these values using the graphic method of Miller and Tainter (Proc Soc Exp Biol and Med 57: 261, 1944). If one half the  $L(ct)_{50}$  can be assumed as a safe concentration, 10 mgm /l (3000 p.p.m.) should be the threshold concentration for an 8 hour exposure in mice. Effects in mice upon vapor inhalation resembled that of other anesthetic agents, e.g., hyperexcitability and ataxia preceding anesthesia. If the exposure was sufficiently prolonged, death ensued due to respiratory failure. The only involvement seen in mice upon autopsy was lung congestion. These same general effects and a similar order of toxicity have been reported for 1,3 butadiene by Carpenter et al (Jour Ind Hyg and Toxicol 25: 255, 1944). Local effects upon application of the undiluted material to the shaved, non abraded skin of the rabbit consisted of immediate vasodilation, with a subsequent erythema which persisted for 24 hours, drying of the skin and superficial sloughing occurred after 5-7 days. The undiluted material applied to the eye of the rabbit caused a conjunctivitis that persisted for 24 hours but was not followed by permanent damage.

The anti-fibrillation action of papaverine and its value in cardiac resuscitation after chloroform-adrenaline ventricular fibrillation K I MELVILLE *Dept of Pharmacology, McGill University, Montreal, Canada* Dogs anesthetized with sodium pentobarbital were used. Artificial respiration was

maintained throughout all experiments by means of a Starling pump. Blood pressure and electrocardiograms (Lead II) were recorded. In normal and vagotomized animals, the intravenous injection of 5 to 10 mgm per kgm of papaverine hydrochloride during chloroform administration, can protect the heart and prevent the development of ventricular fibrillation following intravenous injection of 0.02 mgm per kgm of adrenaline. This protective effect wears off in 20 to 30 minutes, but can be repeated. After induction of chloroform-adrenaline ventricular fibrillation in 12 experiments, the intracardiac injection of papaverine (5 mgm per kgm) in conjunction with cardiac massage, arrested the fibrillation and restored a normal coordinated ventricular beat. At this stage, the heart is dilated, the beat is feeble, and the ventricular rate considerably slowed. Injection of adrenaline (intracardiac) with brief massage of the heart, can then accelerate the heart rate and restore the circulation. This procedure might be of some aid in cardiac resuscitation after ventricular fibrillation. It is suggested that the anti-fibrillation action of papaverine is due primarily to its coronary dilator action. Finally, repeated injections of papaverine during chloroform inhalation can induce circulatory failure associated with ventricular extrasystoles and fibrillation. This condition can however be restored to a normal coordinated beat by cardiac massage.

The effect of some glycerin esters upon the blood pressure, uterus, and respiration RAFAEL MENDOZA and ERNESTO SORI (by invitation) *From the Department of Physiology and Pharmacology of the National Institute of Cardiology, Mexico City* The effects were studied of the acetyl esters of glycerin, monoacetin, diacetin and triacetin and the sulfesters trisulfoglycerin ( $C_3H_5O_12S_3$ ) and tetrasulfodiglycerin ( $C_3H_5O_8S_4-O-C_2H_5O_8S_2$ ) upon the blood pressure, uterus "in situ" and respiration. Eviscerated, adrenalectomized cats under Dial anesthesia were used. Monoacetin up to 0.25 cc/kg caused a small and brief decrease (12-20 mm Hg) in blood pressure with an uncertain effect upon the uterus and an increase in respiration. The same doses of diacetin caused a profound decrease in blood pressure (80-100 mm Hg) lasting 3-5 minutes, a relaxation of the uterus lasting 10-15 minutes and an increase in the rate and amplitude of respiration. Triacetin, 0.05-0.1 cc/kg caused the same effects as Diacetin (0.25 cc/kg). Thus the blood pressure decreasing effect increases with acetylation of the hydroxyl groups of glycerin. Trisulfoglycerin and tetrasulfodiglycerin in doses of 15 mg/kg caused a pronounced increase in blood pressure lasting for 10-15 minutes, and a decrease in the rate and amplitude of respiration. These experiments were conceived to find in the glyceryl radical an effect which would explain the high

potency of nitroglycerin as compared with the heavier organic "nitrates" and with sodium nitrite. The results with the acetines support this assumption. Work is continued with other esters. The results with the sulfo-esters do not invalidate the idea, as the introduction of the  $\text{SO}_2$  usually cancels or modifies the effect of the original compound (Rojahn and Giral, *Productos Químicos Farmacéuticos*, Editorial Atlanta, México, 1942).

**Comparison of digitalis glycosides with their genins in man.** WALTER MODELL, NATHANIEL T. KWIT (by invitation), CONRADO DAYRIT (by invitation), S. J. SHANE (by invitation), and HARRY GOLD, *Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York*. There are some reports in animals showing that the aglycones of the digitalis series possess the typical action of the glycoside on the heart, but that with the removal of the sugar fraction from the glycoside, the potency is usually reduced, the persistence of action is markedly shortened, and a convulsant action may become prominent. How these matters stand in the human subject was tested in patients with congestive failure and auricular fibrillation, using the changes in apex rate as the chief guide. The method was similar to that described in several of our previous studies comparing digitalis materials in man. The final analysis depends on the results obtained in 6 patients in each of whom genins were compared

with corresponding glycosides by intravenous injection, digitoxigenin with digitoxin, digoxigenin with digoxin and lanatoside C. The single doses varied from 0.75 to 2 mg. The genins behave like their glycosides in slowing the ventricular rate in patients with auricular fibrillation. As has been shown in animals, in man also their action on the heart is conspicuously weaker when equal doses by weight are used, the difference being markedly increased when the compounds were compared on a molecular basis. There are 2 other differences, the genins develop their full action within a few minutes as against several hours for the corresponding glycosides, the effect of the genins disappear within 30 minutes to a few hours while that of the corresponding glycosides lasts from many hours to many days.

**Pharmacologic studies of the neurotoxic properties of streptomycin.** HANS MOLITOR and SAMUEL KUNA (by invitation), *Merck Institute for Therapeutic Research, Rahway, N. J.* Even pure streptomycin produces certain toxic manifestations among which a selective effect upon the vestibular system is outstanding. The latter may be regarded, therefore, as an intrinsic property of the streptomycin molecule. Clinical reports indicate, however, that patients receiving equal doses of different

samples of streptomycin may show variations in neurotoxic reactions greater than explainable by differences in individual patient sensitivity. We have previously shown that streptomycin lots causing a high incidence of clinical neurotoxicity also were highly neurotoxic when injected intracisternally into rabbits. However, since the reaction following intracisternal injection of a drug is not necessarily the same as that produced by repeated intramuscular injections (the usual mode of streptomycin administration in man) we compared in rabbits and cats the effect of single intracisternal injections with that of repeated subcutaneous injections. Twenty streptomycin samples ranging in potency from 85 units/mg to 800 units/mg were examined in some 200 cats and 500 rabbits. The doses employed were from 750–1000 units/kg intracisternally and from 25,000–900,000 units/kg/day intramuscularly. Samples which were highly neurotoxic upon single intracisternal injection also possessed high neurotoxicity when given repeatedly subcutaneously. Thus, a sample of streptomycin hydrochloride producing neurotoxic signs upon intracisternal injection of 500 units/kg produced similar signs within 20 days upon daily subcutaneous injection of 50,000 units/kg while a sample with an acute neurotoxicity of 2000 units/kg required from 60 days upward for a comparable effect.

**Rate of disappearance from the blood and distribution in tissues of 3-(orthotoloxyl)-1,2-propanediol (myanesin).** JAMES L. MORRISON, ARTHUR P. RICHARDSON and HARRY A. WALKER (by invitation), *Department of Pharmacology Emory University, Georgia*. Using the diazotization method of Titus, Ulick, and Richardson (*Journ. Pharmacol. & Exper. Ther.* (in press)) the plasma levels of 3-(orthotoloxyl)-1,2-propanediol (myanesin) were determined in a series of dogs infused with varying concentrations of the agent. Blood levels were followed until disappearance from the blood of an intravenous priming dose of 60 mgm/kgm. In most cases the same dogs were used again, after suitable intervals, for intravenous infusion using a priming dose of 60 mgm/kgm followed by the infusion with myanesin at the rate of 1 mgm/kgm/min for two hours. Subsequently this was repeated using a rate of infusion of 2 mgm/kgm/min. Blood levels were determined at suitable intervals during infusion and until the drug disappeared from the blood stream on cessation of the infusion. Infusion was through a cannula in the femoral vein. Blood samples were taken from the jugular vein. Following the last infusion certain animals were sacrificed by injection of air and organs were removed and myanesin content was determined by the above method. Concentrations of myanesin appear to be much higher in brain, pancreas, kidney, spleen, in a descending order and



only slightly higher than plasma in muscle, lung and liver. The concentration of myanesin in spinal fluid and saliva was slightly lower than that in plasma. Blood level curves obtained by this method indicate that doses as high as 60 mgm /kgm can be disposed of by the body in a matter of 30-60 minutes. By infusing with myanesin after such a dose of 60 mgm /kgm an infusion rate can be estimated which will maintain a constant level in the blood. Since infusion rates of 1 mgm /kgm /min will not maintain a constant level and rates of 2 mgm /kgm /min leads to accumulation of myanesin the maintenance level must lie somewhere between.

**Studies with the polyoxyethylene derivatives of sorbitan partial esters (Tweens)** RUTH MUSSER (introduced by JOHN C KRANTZ, JR.) *Department of Pharmacology, Univ of Maryland, School of Medicine, Baltimore* Tween 20, 40, 60 and 80 were studied in relation to their capacity to hemolyze red cells. Although the Tweens as a class were found to be hemolytic, their products of hydrolysis were nonhemolytic. Pancreatic lipase caused the hydrolysis of Tweens *in vitro*. The rate of the polyoxyethylene derivative of sorbitan was studied in humans with special reference to excretion and exhalation.

**Effect of procaine on cyclopropane-epinephrine cardiac arrhythmias** MARK NICKERSON *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah* After pentothal induction, dogs were equilibrated to a 30 per cent cyclopropane-70 per cent O<sub>2</sub> anesthetic mixture for 30 minutes and then tested with an intravenous dose of 10 µgm /kgm epinephrine. Procaine HCl (10 mgm /kgm) administered 10 minutes prior to testing provides only slight protection against the epinephrine induced cardiac irregularities. However, the same dose of procaine injected rapidly after irregularities develop will stop them as soon as the drug reaches the myocardium. Reversion to a sinus rhythm is accompanied by a prolongation, a reduction in amplitude and other changes in the QRS complexes of the EKG indicating altered myocardial conduction. Intravenous administration of 10 mgm /kgm procaine HCl over a period of one minute considerably reduces the maximum frequency of A-V impulse transmission (reciprocal of the physiologically effective refractory period). Maximum reduction occurs near the end of the injection while a high concentration of procaine is being carried directly to the heart. Less than 40 per cent of the effect remains 3 minutes after the injection and it is almost completely gone within 10 minutes. The above results indicate that high concentrations of procaine are necessary to suppress epinephrine induced ectopic rhythms. They provide an explanation for the reported efficacy of procaine in arresting cardiac arrhythmias during

anesthesia, but point to a very limited prophylactic value of this agent.

**Dibenamine protection against cyclopropane-epinephrine cardiac arrhythmias** MARK NICKERSON and GEORGE M. NOMAGUCHI (by invitation) *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah* Three etiological factors proposed to explain cardiac irregularities produced by epinephrine in the presence of anesthetics are: (1) a direct effect of suddenly increased arterial pressure upon sensitized ventricles, (2) an inhibitory vagal action (secondary to increased arterial pressure) on the S-A node during the period of direct myocardial stimulation by epinephrine, and (3) a direct effect of epinephrine on the sensitized myocardium. All of these factors could be altered by Dibenamine. The nearly complete protection afforded by Dibenamine against cyclopropane epinephrine arrhythmias is not diminished by constriction of the thoracic aorta in such a way as to reproduce the pressor effect characteristic of epinephrine in the absence of Dibenamine. The protection is thus unrelated to blocking of the epinephrine-induced rise in arterial pressure. Although strong electrical stimulation of the vagus produces a ventricular rhythm in the presence of both epinephrine and Dibenamine, the vagus is probably not an important factor in cyclopropane epinephrine arrhythmias because bilateral vagotomy is not protective and reflex activation of the vagus does not alter Dibenamine protection. Dibenamine, therefore, appears to protect by acting directly on cardiac muscle, but its exact mode of action is still obscure. It does not have any direct effect on the heart detectable in the EKG, and does not alter the cardioaccelerator or inotropic effects of epinephrine. In addition, its "quinidine-like" effect on impulse conduction in cardiac muscle is too short in duration to account for the protection, and is also exerted by congeners which do not protect against cyclopropane epinephrine arrhythmias.

**Some applications of sequential analysis** R. H. NOEL and M. A. BRUMBAUGH (introduced by LLOYD C. MILLER) *Bristol Laboratories, Inc., Syracuse, New York* Sequential analysis of laboratory results offers two major advantages to the technician, (a) defined risks of erroneous decisions, (b) reduction of the number of experiments required to arrive at decisions. Three illustrations are presented in this paper: (1) The amount of testing required to determine whether an experimental drug is superior to an established preparation. (2) The amount of testing required to determine whether a new antibiotic strain is superior to an established one. (3) The amount of testing required to assess the relative value of two assay methods.

The renal elimination of caronamide (4'-car-

**boxyphenyl-methanesulfonanilide** HAROLD M PECK, HORACE F RUSSO, ELIZABETH K TILLSON, WILLIAM S WALLER (all by invitation) and KARL H BEYER *From the Department of Pharmacology, The Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa* Caronamide (4'-carboxyphenylmethanesulfonanilide) is excreted as such and in an "altered", probably conjugated, form. The analytical methods of Sprague and Ziegler (J Lab Clin Med, In press) and of Brodie, Levy and Bernstein (J Pharmacol 91 246, 1947) have been used in these experiments. Although these methods are reasonably accurate, the fidelity with which they measure "unaltered" caronamide (method of Brodie, et al) or total caronamide (method of Sprague and Ziegler) in urine or plasma of an animal to which the drug is administered does not appear to be certain. This increases considerably the desirability of several approaches to the problem of renal elimination of caronamide and the necessity for caution in interpreting the results. The results of the following types of experiments relating to the renal extraction, clearance, and urinary recovery of caronamide will be presented: 1) A comparison of the falling plasma concentration and urinary recovery of "unaltered" and "total" caronamide with mannitol; 2) The renal clearances of "unaltered" and "total" caronamide calculated on the basis of whole and ultrafiltered plasma; 3) A correlation of the extraction of "unaltered" and "total" caronamide and creatinine from the renal circulation with simultaneous renal clearances of the compounds on the basis of whole and ultrafiltered plasma.

**Synthetic curare compounds II** d-N-methyl-isochondrodendrine iodide and d-N-methyl-O-methyl-isochondrodendrine iodide M H PELLETIER (by invitation) and DAVID FIELDING MARSH *Department of Pharmacology, West Virginia University School of Medicine, Morgantown, W Va* d-Isochondrodendrine was isolated from an old sample of "Bebeerine Pure" and from the roots of Chondrodendron tomentosum. The curariform activity of its derivatives, d-N-methyl isochondro-

dendrine iodide (iCM), and d-N-methyl O-methyl-isochondrodendrine iodide (iCMMeO) was determined in comparison with the corresponding isomers, d-tubocurarine chloride pentahydrate (dT), and d-O-methyl-tubocurarine iodide trihydrate (dTMeO).

Like the tubocurarine compounds, these isochondrodendrine derivatives have relatively little effect in intact animals other than lissive action on skeletal muscles.

**Chronic intoxication of the CNS produced by the methadon side-chain** CARL C PFEIFFER, ELIZABETH H JENNY (by invitation) and I GERSH *Depts of Pharmacology and Pathology, Univ of Illinois College of Medicine, Chicago 12* The methadon side-chain, dimethyl, 2-chloropropylamine HCl, produces a non-lethal type of persistent ataxia when injected into mice or rats in a dosage range of 100 to 200 mgm/kgm. The LD 50 after intraperitoneal injection in the mouse is  $231 \pm 17$  mgm/kgm, slope 7.1. The volatile compound dimethyl, 2-chloropropylamine produces a similar syndrome in rats when concentrations as low as 10 parts per million are inhaled for 15 minutes. Brain specimens were collected from intoxicated rats after carotid perfusion with saline and 10 percent neutral formalin. Celloidin sections of cerebrum and cerebellum were made and stained with Toluidine Blue and Hematoxylin and eosin. Microscopic examination showed no changes in the cerebrum. Pathological changes in the cerebellum consisted of petechial hemorrhages in the gray and white matter, hyperchromatism and shrinkage of the Purkinje cells and mobilization of the glial cells. One or more of these lesions was present in all specimens examined. Similar CNS ataxias have been described after the administration of other chlorinated amines (Krop, S, et al Fed Proc, 6 347, 1947; A Goldin et al Bib Sci and Ind Rept, 5 678, 1947) and also after chlorinated anesthetic agents (Abreu, B E, et al Anesth and Analg, p 131, May-June 1939). It is concluded that workers in chemical industry should avoid exposure to these volatile amines.

**The nature of the prosthetic groups of analgesics and their possible action as blocking agents** CARL C PFEIFFER, J SANTOS-MARTINEZ (by invitation) and THEODORE R SHERROD (by invitation) *Department of Pharmacology, University of Illinois College of Medicine, Chicago 12, Ill* Studies on potent analgesic drugs, using Hirschfelder atomic models, suggest that analgesic drugs owe their pharmacological activity to three factors: 1) a methyl on tertiary nitrogen prosthetic group (quaternization does not enhance the analgesic action of methadon), 2) the presence of several active oxygen prosthetic groups at a distance of 7.0 to 9.0 Angstroms from the methyl on nitrogen group, and 3) the presence of one or more

|                          | dT           | dTMeO         | iCM       | iCMMeO     |
|--------------------------|--------------|---------------|-----------|------------|
| Albino rats              |              |               |           |            |
| LD 50                    | 0.27 (0.22)* | 0.032 (0.022) | 5.5 (3.9) | 3.0 (2.2)  |
| Rabbits                  |              |               |           |            |
| Head drop 50             | 0.12 (0.10)  | 0.016 (0.011) | 2.5 (1.8) | 0.6 (0.4)  |
| Holiday head drop        | 0.15 (0.12)  | 0.020 (0.014) | 2.9 (2.1) | 0.7 (0.5)  |
| LD 50                    | 0.35 (0.28)  | 0.040 (0.027) | 4.5 (3.2) | 1.3 (0.9)  |
| Cat gastrocnemius muscle |              |               |           |            |
| Equivalent paralysis     | 0.05 (0.04)  | 0.007 (0.005) | 1.0 (0.7) | 0.2 (0.14) |

\* All doses given in milligrams/kilogram body weight. Dose of equivalent contained curariform ion given in parenthesis.

blocking moieties (phenyl, biphenyl, or dibutyl). The relationship of the prosthetic groups is independent of the general ring structure and obtains for the phenanthrenes, biphenyls, piperidines, oxazolidinones, etc. Since these prosthetic groups are similar to those of the acetylcholine-atropine series, compounds such as meperidine (demerol) which have a shorter distance between prosthetic groups than that postulated for the potent analgesic drugs, show decreased analgesic action and varying degrees of atropine like action. Assuming that these analgesic drugs depress the CNS by blocking a specific CNS metabolite, the following known metabolites and their derivatives have been studied to determine if any will lower the normal pain threshold. The known amino acids studied are glutamic acid, lysine, d arginine, ornithine, and l-tryptophane. Synthetic derivatives have also been synthesized and their action will be reported.

**Use of hyperglycemic response for estimating addiction potentialities of analgesic compounds**  
NILKANTH M. PHATAK, JAMES MALONEY (by invitation) and NORMAN DAVID, *Department of Pharmacology, University of Oregon Medical School, Portland, Oregon*. An objective evidence of morphine habituation was observed by Ro (Jap J Med Sci IV Pharmacology, 9: 59, 1935). He noted that during development of tolerance to morphine in animals, hyperglycemic responses to successive injections become progressively less but upon withdrawal a hyperglycemic response appears at the expected time of injection. In a later biochemophologic study of morphine derivatives, Emerson and Phatak (Univ Calif Publicat in Pharm 1: 77, 1938) confirmed Ro's observations and suggested that the hyperglycemic withdrawal phenomenon might be useful for estimating addiction potentialities. The present study compares hyperglycemic responses to lamidone (Merck), dl amidone (Dolophine, Lilly), dl isoamidone (Merck), meperidine (Demerol, Winthrop) and morphine in rabbits, using saline controls. Initial injections of all analgesic compounds produced hyperglycemia. The animals were injected daily for a period of six weeks, with weekly increments in dosage as follows: amidone compounds, 5, 6, 7, 8, 9 and 9 mg/kg; morphine sulfate, 10, 20, 30, 40, 50 and 60 mg/kg; meperidine HCl, 50, 75, 90, 100, 110 and 110 mg/kg. During development of tolerance greatest hyperglycemic responses followed injections of l amidone and dl amidone. During the withdrawal period the greatest hyperglycemic response occurred with l amidone, was slightly less following morphine and dl amidone, and negligible for dl isoamidone and meperidine. Convulsions, opisthotonus and a cataleptic state with temporary respiratory arrest followed injection of the synthetic compounds l-Amidone and

dl amidone produced these signs at the initial dosage of 5 mg/kg, while those noted for dl isoamidone were less marked and did not appear until the dosage reached 8 mg/kg. Meperidine produced the same signs at 90 mg/kg.

**Studies on the toxicology of podophyllotoxin and related substances**  
FREDERICK S. PHILLIPS, MAYNARD B. CHINOWETH, and CARLTON C. HUNT (by invitation). *From the Departments of Pharmacology, Cornell Univ. Medical College and the Sloan Kettering Institute for Cancer Research, New York, N. Y.* Recent demonstrations of colchicine-like effects of podophyllotoxin and podophyllin in arresting cell-mitosis in metaphase and actions of the drugs against *condylomata acuminata* and experimental tumors stimulated studies of their toxicology. Intravenous LD<sub>50</sub>'s of podophyllotoxin were: rats, 87; cats, 17; rabbits, ca. 5 mg/kg; intraperitoneally mice, 33; rats, 15 mg/kg; intramuscularly cats, ca. 4; rats, ca. 3 mg/kg. Cats and dogs receiving seven or more daily, intravenous doses of 0.5 mg/kg of podophyllotoxin survived without alarming consequences. Similarly mice tolerated 8 mg/kg daily intraperitoneally. In confirmation of previous investigations podophyllin was found to contain toxic components not identical with podophyllotoxin. Several residues remaining after solvent extraction of podophyllotoxin from podophyllin proved to be equally or even more toxic in rats and mice than podophyllotoxin itself. Animals receiving fatal doses of podophyllotoxin usually succumbed within 24 hours. Initial signs included emesis, defecation with tenesmus, and respiratory stimulation. Dogs occasionally developed bradycardia and ventricular extrasystoles (reversed by atropine). Muscular weakness, heralded by hind leg ataxia, resulted in eventual prostration. Terminal respiration was slow and labored, blood pressure fell to shock levels, and death followed respiratory failure. In cats and rats, but not in dogs, fatal doses often caused severe pulmonary damage. A small proportion of animals receiving minimum lethal doses exhibited progressive cachexia and succumbed after several days. These fatalities were not associated with gastro intestinal, hepatic, or renal damage or disturbances of hematopoiesis. However, toxic doses did elicit a marked granulocytopenia within 1 hour after administration from which animals recovered promptly.

**Spasmolytic and other pharmacologic properties of  $\beta$ -piperidinoethyl phenyl- $\alpha$ -thienylglycolate**  
RAYMOND W. PICKERING and RICHARD C. BURNETT (introduced by BENEDICT E. ABREU). *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*.  $\beta$ -piperidinoethyl phenyl- $\alpha$ -thienylglycolate (P.P.T.) in doses ranging from 0.4 mgm/kg to 8 mgm/kg, subcutaneously produced spas-

molysis, lasting from 1 to 62 minutes in 18 monkeys whose colons had been rendered hyperactive with morphine Triasentin in doses as high as 10 mgm / kg, subcutaneously did not produce comparable effects In preliminary experiments on anesthetized dogs, P P T 25 mgm /kg I V, depressed the activity of the ileum and jejunum for 122 minutes P P T usually produced spasm in the non pregnant guinea pig uterine strip in concentration of  $5 \times 10^{-3}$  mgm /ml occasionally relaxation was observed at this concentration In 33 human subjects, P P T did not produce any untoward effects in doses ranging from 25 mgm to 35 mgm given orally One subject who received 35 mgm complained of vertigo, drowsiness, dimness of vision and chills for 10 minutes after ingestion Complete recovery occurred within 60 minutes In other subjects lower doses did not produce hypnotic or analgetic effects, central nervous system stimulation, cycloplegia, mydriasis, xerostomia, nor changes in respiration, blood pressure, electrocardiographic tracings nor cardiac rate Examination of urine revealed no abnormalities In 4 subjects given 5 and 10 mgm sublingual doses of P P T, spasmolysis for an average of 36 minutes (30-62 minutes) occurred There was no increase in gastric acidity upon sublingual administration of P P T to 4 individuals

**Abolition of extra systoles in the perfused isolated rabbit heart by procaine and monocaine hydrochlorides** HARRY J PRATT (introduced by R BEUTNER) *Department of Pharmacology,ahnemann Medical College, Phila, Pa* In connection with the study of the cardiac toxicity of Procaine and Monocaine Hydrochlorides on the perfused isolated rabbit heart, it was observed that extra systoles were always shown by the normal rabbit heart during the control period Following the addition of Procaine or Monocaine in a concentration of 1 100,000 to the perfusion fluid, complete abolition of extra systoles was observed in 100% of the experiments Monocaine Hydrochloride in a concentration of 1 150,000 in the perfusion fluid prevented the continuance of extra systoles in about 50% of further experiments and at a concentration of 1 500,000, no effect upon the prevention of extra systoles was observed Lower concentrations of Procaine Hydrochloride were not used These findings are in agreement with the clinical use of intravenous administration of local anesthetics in cardiac irregularities elicited by Cyclopropane anesthesia or from other causes, since they demonstrate the regularizing influence of local anesthetics upon the heart

**Toxicological properties of the causative agent of canine hysteria** JACK L RADOMSKI, GOEFFREY WOODARD, CARTER D JOHNSTON (introduced by ARNOLD J LEHMAN) *Division of Pharmacology, Food and Drug Administration, Federal Security*

*Agency, Washington 25, D C* Recent work in this and other laboratories has established that a reaction product of nitrogen trichloride ( $\text{NCl}_3$ ) with the protein of wheat flour is the etiological agent of canine hysteria The cumulative characteristics of this toxic agent have been investigated Several groups of 10 month old dogs were fed  $\text{NCl}_3$ -treated gluten (6 gm  $\text{NCl}_3$ /kg gluten) in doses ranging from 25 to 0.1 gm per kg per day When the average number of days required for the animals to come down with running fits on each dosage level was plotted against the daily dosage, a hyperbola like curve was obtained This curve becomes asymptotic at about 0.1 gm /kg /day Apparently the dog is capable of destroying and/or excreting the amount of toxic principle contained in about this amount of  $\text{NCl}_3$  treated gluten Translating these data into other terms, it means that a dog could consume normally treated flour (15 gm of agene/100 lbs) to the extent of  $\frac{1}{4}$  of his diet without developing toxic effects The sensitivity to constant dosages of treated gluten per kilogram of body weight varies with the age of the dog, 12 month old dogs being more sensitive than 6 month old dogs Differences in sex susceptibility have not been observed Various drugs including those used in epilepsy have been tested for their value in canine hysteria

**The effect of cyclopropane on the work capacity of the dog heart** BARBARA RIVICK (by invitation), S DONALD MALTON (by invitation), and GORDON K MOL *From the Department of Pharmacology, the University of Michigan, Ann Arbor* It has been established that the cardiac output of animals under cyclopropane anesthesia is maintained at normal levels until concentrations producing respiratory arrest are reached It has been assumed that in anesthetic concentrations the gas produces no impairment of cardiac function It is, however, impossible to estimate the effect of a drug on cardiac reserve by measurement of cardiac output alone, since this may be maintained within relatively normal limits in the presence of severe limitation of cardiac work capacity In heart-lung preparations exposed to various concentrations of cyclopropane increased work loads were offered by increasing arterial resistance, responses were measured in terms of auricular pressures and cardiac output In intact animals the work load was increased by elevating arterial pressure with the aid of a pressure regulator attached to the abdominal aorta, and the responses were estimated in terms of right auricular pressure changes After the responses to graded pressure loading were measured during exposure to various concentrations of cyclopropane, sodium pentobarbital anesthesia was substituted Artificial respiration was maintained throughout The work capacity of the isolated heart was significantly reduced by 20% cyclo-

propene, and greatly reduced by 10%. In the intact animal the elevation of venous pressure in response to pressure loading was significantly greater under 20% cyclopropene than with 30 mgm/kg of pentobutyl, and markedly greater under 10% cyclopropene than with 15 mgm/kg of pentobarbital. We conclude that cyclopropene in anesthetic concentrations diminishes the work capacity of the dog heart.

**Detoxification of 2-benzyl-imidazoline hydrochloride (Priscol) by incubation with hepatic slices from the rat.** B. RICHARDS (by invitation), A. CAMERON (by invitation), B. CRAVER, E. HERROLD (by invitation). *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey.* Two methods were employed to determine whether 2-Benzyl-Imidazoline Hydrochloride was detoxified "in vitro" by incubation with hepatic slices from the rat. An investigation of the toxicity of various imidazolines upon isolated tissues first led to these studies of detoxification. By means of the Warburg technique, it was found that a concentration of 10 mg/ml of Priscol caused little or no inhibition of the respiratory rate of hepatic slices from the rat but a considerable decrease in the respiration of renal slices from the rat. The technique of Finklestein, as described elsewhere in the Proceedings, was employed with an extension to include non cardiac tissues. A dose of 10 mg/ml of Priscol, after incubation with hepatic slices from the rat, proved less toxic to renal slices from the rat. We interpreted this to indicate hepatic detoxification. In the second method the drug was incubated with hepatic slices from the rat and the Priscol subsequently bioassayed on the isolated, perfused heart of the guinea pig. Priscol in small doses exerts a positive inotropic action on this heart. Priscol, first incubated with liver, is much less active in its cardiac effect than Priscol not so incubated. The minimally toxic doses used in these experiments far exceeded any level that would be attained therapeutically. These experiments confirm those of others indicating the feasibility of this approach to detoxification.

**Further analysis of the influence of autonomic innervation on drug responses of the heart and gut in unanesthetized dogs.** JAMES A. RICHARDSON (by invitation) and R. P. WALTON. *Dept of Pharmacology, Medical College of South Carolina.* Heart slowing in dogs, as produced by morphine (10 mgm per kgm subcutaneously) was not present during the few days survival interval after bilateral cervical vagotomy. Morphine induced gut spasm as determined by Thierry fistula tracings was, however, obtained during a 36 hour interval after cervical vagotomy and during a 6 weeks interval after supra diaphragmatic vagotomy. (Morphine test doses were either 0.5 or 1.0 mgm per

kgm subcutaneously.) Also following vagotomy at the diaphragm level, acetylcholine effects on gut movements were essentially unchanged in some cases and moderately intensified in others. (Acetylcholine administration before and after vagotomy was effected by intravenous infusion at a uniform rate of 0.2 mgm per kgm per minute for 5 minutes.) High spinal anesthesia with procaine had a consistently depressant effect on the typical Thierry fistula tracing. Dibenamine in doses up to 10 mgm per kgm intravenously either failed to affect the character of the typical Thierry fistula tracing or produced moderate increase in general tone level, following these dibenamine injections, characteristic epinephrine depressant effects and characteristic morphine stimulant effects were obtained.

**Elevation of serum protein-bound iodine after large doses of radio-active iodine.** DOUGLAS S. RIGGS (introduced by OTTO KRAEYER). *Department of Pharmacology, Harvard Medical School and The Thyroid Clinic, Massachusetts General Hospital.* Shortly after the administration of therapeutic doses of radio active iodine to hyperthyroid patients, a transient increase in metabolic rate, swelling and tenderness of the thyroid, and radiation sickness have occasionally been noted. None of these reactions has been alarming. However, Saltz has described a marked elevation of plasma protein bound iodine and the development of hyperthyroidism when a large dose of radio active iodine was given to a patient with massive metastases from a functional carcinoma of the thyroid gland. Additional instances of such elevations are reported here. The protein bound iodine of a patient with thyroid carcinoma rose from 4.3 to 15.0 micrograms per cent 7 days after a "thyroidectomizing" dose of  $I^{131}$ . A significant, though smaller, increase was also observed in another patient with thyroid carcinoma and mild hypothyroidism. These two patients were in the Sloan Kettering Institute. The protein bound iodine of a patient with Graves' disease rose from 16.2 to 23.0 micrograms per cent 7 days after a therapeutic dose of  $I^{131}$ . A similar increase occurred in a patient with severe hyperthyroidism, but it could not be ascribed conclusively to therapy with radioactive iodine. This patient died of coronary occlusion 19 days after treatment. The transient rise in serum protein bound iodine is presumably due to a sudden release of preformed hormone from the gland to the blood stream when the thyroid is exposed to intense radiation. These observations suggest that large doses of radioactive iodine may precipitate an acute exacerbation of hyperthyroidism in certain patients.

**The effect of globin insulin and protamine zinc insulin on the diurnal blood sugar curve and the daily glycosuria of diabetics, and the clinical use**

**of globin insulin** JOSEPH THOMAS ROBERTS *From The University of Arkansas School of Medicine, Little Rock, Arkansas, and Gallinger Municipal Hospital, Washington, D C* Globin insulin controlled the average blood sugar curve and duly glycosuria of 97 diabetics in a more nearly normal way than did the same doses of protamine zinc insulin With globin insulin, the three preprandial and midnight blood sugar levels had normal average values, while with protamine zinc insulin in equal doses only the fasting im value was normal With globin insulin, the three postprandial rises in blood sugar levels were smaller than with the other preparation Globin insulin was effective in maintaining good control of diabetics in the out patient clinic, and seemed of some value in smoothing the recovery curve of blood sugar values of diabetic acidosis when used in conjunction with regular (crystalline) insulin Local and systemic reactions were fewer and milder with globin insulin than with protamine zinc insulin Serious reactions with globin insulin may follow ignoring the precautions generally used with protamine zinc insulin

**Comparative intragastric and local toxicities of Tert-butyl-hydrogen peroxide, di-tert-butyl-peroxide, and 2,2 bis-di-tert-butyl-peroxybutane** R R ROLLINS (introduced by HAMILTON H ANDERSON) *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco* Tert-butyl hydrogen peroxide has been reported to have biologic activity (Mendex, R, Jour Pharmacol, 82 377, 1941) it is compared here to two of its derivatives and to ascaridol The peroxides were administered to rats and mice intragastrically, either undiluted or in cottonseed oil (see table) The percentage purity of the compounds is indicated The lower boiling

on inhalation are probably related to differences in boiling points The more volatile TBHP and TBP killed in less time because of higher vapor concentrations The tert butyl hydrogen peroxide was the most irritant of the three compounds when applied topically In one individual, dermatitis venenata developed after brief contact 2,2 bis di-tert-butyl peroxybutane had a similar action on the skin and eye of the rabbit as butyl acetate when compared with previously reported results (McOmie and Anderson—to be published)

Preliminary studies of ascaricidal activity (*in vitro*) indicated that only tert butyl hydrogen peroxide had an activity of the same order as ascaridol

**Adenylpyrophosphatases and other phosphatases in the cell surface of living yeast** ASER ROTHSCHILD (by invitation) and REBECCA MEIER (by invitation) (introduced by HARVEY B HAAG) Various phosphate compounds such as adenyln triphosphate (ATP), adenyldiphosphate (ADP), Na glycerophosphate, inorganic triphosphate, or inorganic pyrophosphate, added to a suspension of washed, starved living Bakers' yeast (Standard Brands) are hydrolyzed with the production of inorganic ortho phosphate An extensive study of muscle and yeast ATP breakdown indicates that during hydrolysis, the products (adenylic acid and inorganic phosphate in the ratio of 1:2) and unused substrate can be recovered quantitatively from the medium In addition, if ATP containing radioactive phosphate ( $P_{32}$ ) is hydrolyzed by the cells, all of the  $P_{32}$  is recovered in the medium with none in the cells These experiments indicate that the enzyme is at or in the cell surface However, the enzyme is firmly bound, because the medium separated from the cells shows no activity A study of the pH-activity curves and of competition or non competition of various substrates with each other, indicates that ATP, ADP, and inorganic triphosphate are hydrolyzed by an enzyme with a pH optimum of 3.5 and almost zero activity at 7.0 and higher The Na glycerophosphate and inorganic pyrophosphate are hydrolyzed at a lower rate by other enzymes which are active at pH 6.0 and below but with no well defined optimum The limiting substrate concentration for the ATP hydrolysis is about  $0.7 \times 10^{-3}$  M With optimum ATP concentrations the rate of hydrolysis is directly proportional to yeast concentration, indicating a first order reaction The time course of hydrolysis, however, follows an asymptotic curve rather than a linear function due not to destruction of enzyme, but apparently to inhibition by the products of the reaction, inorganic phosphate and adenylic acid A synergistic effect between these two substances seems to be involved The inhibition due to the phosphate is greatly increased by the adenylic acid, which by itself has no effect

| Compound                                  | Approximate purity | No of rats | No of mice | Estimated LD <sub>50</sub> , ml/kg |      | Time to kill 50% of mice (sat vapor air mixture) |
|---|--------------------|------------|------------|------------------------------------|------|--|
|   |                    |            |            | Rats                               | Mice |  |
| Ascaridol                                 | 85%                | 22         | 12         | 0.20                               | 0.40 | Undetermined                                     |
| tert butyl hydrogen peroxide (TBHP)       | 60%                | 29         | 14         | 1.5                                | 2.0  | 10-15 minutes                                    |
| di-tert-butyl peroxide (TBP)              | 90%                | 17         | 12         | 17.5                               | 20.0 | 10-15 minutes                                    |
| 2,2 bis di-tert-butyl peroxybutane (TBPB) | 80%                | 24         | 36         | 12.5                               | 17.5 | more than 510 minutes                            |

components of 2,2 bis compound (TBPB) were removed upon aeration The differences in toxicity

**Effect of quinidine on the metabolism of the rat heart** PAUL R. SANDERS (by invitation), J. LEIDEN WELLS (by invitation), and CLYTON H. THURNES *Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles, California* Quinidine at a concentration of 0.0003M was found to depress the endogenous oxygen uptake of rat ventricle slices approximately 50 per cent, and also to decrease the ability of glucose to maintain the oxygen uptake of such slices. This concentration has been found to produce typical quinidine effects on the isolated perfused rat heart. Only slight effects were observed at this concentration on the utilization of succinate, lactate, pyruvate, and malate, either with tissue slices or homogenates, although higher concentrations were somewhat inhibitory to several dehydrogenation reactions. Further evidence has indicated that quinidine exerts its depressant action on rat heart metabolism probably chiefly by inhibition of the earlier stages of glucose breakdown. The effects of quinidine on the breakdown and synthesis of high energy phosphate bonds in rat heart homogenates has also been investigated. It apparently does not markedly affect adenosine-triphosphate breakdown at several low concentrations which depress the isolated perfused heart.

**Use of oxygen consumption method for measuring antithyroid activity of n-propylthiouracil, thiouracil and 2-aminothiazole in rats** EDWARD SAGEY (by invitation) and VILKINATH M. PHATK *Department of Pharmacology, University of Oregon Medical School, Portland, Oregon* Direct measurement of the  $O_2$  consumption rate seems logical in preference to indirect methods now utilized to estimate the relative effectiveness of antithyroid drugs. Using a modified Peoples' Metabolic (Phatak and Sagey, *J. Amer. Pharm. Assoc.*, 36: 105, April, 1947) we have determined weekly  $O_2$  consumption rates in rats before and after treatment with thiouracil, n-propylthiouracil and 2-aminothiazole. The latter compound was fed in drinking water as a 0.1% solution while the thiouracils were injected intraperitoneally in doses of 100 mg/kg per day. The duration of treatment varied from two to four weeks. After administration of n-propylthiouracil the  $O_2$  consumption rate of rats showed a decrease from a mean value of  $20 \pm 4$  cc/min/kg bodyweight to  $19.0$  cc/min/kg at the end of two weeks. Within a comparable period the  $O_2$  consumption rate of the rats given 2-aminothiazole decreased from a normal value of  $19.3$  cc/min/kg to  $17.6$  cc/min/kg with a further decrease to  $16.3$  cc/min/kg at the end of four weeks. Thiouracil at 100 mg/kg dose gave no indication of effectiveness after two weeks of administration.

**The circulation of penicillin in the lymph** R. J. SCHACHTER (introduced by N. ERCOLI)

*Dept. Pharmacology and Chemotherapy, Warner Inst. Therap. Research, New York City* From the work of Romansky (*New England J. Med.* 223: 577, 1945) it is known that penicillin might still be present in the urine after the blood stream apparently is penicillin-free. We found, following an intramuscular injection of a suspension of penicillin in oil with vasoconstrictor, that the duration of penicillin in the tissues is more than twice as long as in the blood stream. An explanation of these observations indicated a follow up of the fate and circulation of penicillin in the body, particularly at the end period of the blood level curve and afterwards. This investigation revealed that the circulation of penicillin in the lymph might be as important a factor in its transport as the blood circulation. As a rule, the lymph collected from the cannulated thoracic duct of dogs contained detectable amounts of penicillin for significantly longer periods than the blood stream. The penicillin present in the lymph—probably collected from the organs—eventually reaches the blood stream where it is diluted to a point beyond detection, then again concentrated in the kidney and eliminated. This explains why the blood apparently is free from penicillin while the kidneys still excrete detectable amounts. The present findings on the prolonged lymphatic circulation, the persistence of penicillin in the organs, and the chemotherapeutic activity without detectable blood levels, observed by Ercoli, Lewis, Schwartz, and Whitehead (this issue), lead to the conclusion that the blood level is only one of many factors related to the effectiveness of penicillin therapy.

**Malaria chemotherapy 2. The response of sporozoite-induced infections with *Plasmodium cynomolgi* to various antimalarial drugs** L. H. SCHMIDT, ROCHELLE FRADKIN (by invitation), WANDA SQUIRES (by invitation), and CLARA S. GENTHER (by invitation) *Christ Hospital Institute of Medical Research, Cincinnati, Ohio* The activities of various antimalarial drugs against trophozoite induced infections with *Plasmodium cynomolgi* were described in the preceding paper (*Fed. Proceedings*, this issue, p. 221). This report deals with response of sporozoite induced infections to quinine, quinacrine, chloroquine, oxychloroquine, chlorguanide (*Paludrine*), pamaquine, pamaquine plus quinine, pentaquine and pentaquine plus quinine. Approximately 250 rhesus monkeys were infected intravenously with measured doses of sporozoites. Treatment with one of the above drugs was initiated either in the ascending phase of the primary attack, or during the first, second or third relapses in cases where infections were not cured by previous medication. In all cases drugs were administered once daily, the duration of treatment being either 7 or 14 days. Criteria of cure were identical with those used in the tropho-

zoite-induced infections (loc cit) Results were as follows Quinine, quinaquine, chloroquine, oxychloroquine, and chloiguanide suppressed the clinical attacks of the sporozoite induced infections in the same doses as were effective against the trophozoite-induced disease Cures were not effected by these drugs, however, even when doses were administered which were 60 times greater than those which cured trophozoite-induced infections In contrast, pamaquine and pentamine, administered either alone or with quinine did effect cures Dose for dose these 8 aminoquinolines appeared to be equally effective These results indicate rather clearly that the chemotherapeutic characteristics of *P cynomolgi* infections in the rhesus monkey are identical with those of *P vivax* infections in man

**Comparison of therapeutic values of several antimonials in experimental schistosomiasis mansoni in mice** MAXWELL SCHUBERT and ELLIAN GOLDBERG (by invitation) *From the Department of Therapeutics, New York University College of Medicine* Methods previously described for production of standard infections of *Schistosomiasis mansoni* in mice, for systematic autopsy of these mice and estimate of the effects of drugs on such infections, had been applied only to rough screening of drugs They have now been used in an attempt to measure therapeutic effectiveness of several antimonials on the disease in mice Two methods are discussed The first is the simple therapeutic index, the ratio of the chronic LD50 to the CD50 where the latter quantity is the dose that frees fifty per cent of the mice completely of worms The second is based on the fraction of worms destroyed Because of the high variability of worms found per mouse even in control groups this fraction is taken as nine tenths rather than one half So the second measure of therapeutic effectiveness is the ratio of the LD50 to the dose that reduces the average worm count to about a tenth of its value in control untreated mice Errors in both of these indexes are discussed in detail Within the wide limits of error to which the values being measured are subject, both indexes have similar magnitudes For sodium antimony tartrate, ureastibamine, neostibosan and neostam the indexes are below one Fuadin is the best of the antimonials currently used, its index is one Two oil soluble antimonials are the only drugs known to have an index greater than one Implications are discussed

**The chemotherapeutic action of a number of furan derivatives** LLOYD D SEAGER, *Department of Pharmacology, The Woman's Medical College of Penna* Twenty furan derivatives were studied for their chemotherapeutic effectiveness against *Trypanosoma equiperdum* infections Mice were inoculated intraperitoneally with 500,000 to 1,000,000 parasites/kg With the strain of *Equiperdum* used this produces 100% fatality in untreated mice

within 5 days Drugs were given by stomach tube 3 times daily for 4 days Soluble compounds were tested also by the single intraperitoneal method Furfural, 5-nitro 2 furildehyde semicarbazone, furalacetate, furfuryl alcohol, furoic acid and dimethyl acrylic acid were found to be active Additive effects but no synergism was found between the active furan derivatives Furfural was found to have additive effects but no synergism with acriflavine, stilbramidine and pentamidine None of the furan compounds tested were found to influence the development of *T cruzi* infections in mice

**The nutritive value of the fatty acids of lard esterified with a polyethylene glycol** C BOYD SHAEFFER and FRANCIS H CRITCHFIELD (introduced by PAUL L McLAIN) *Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh, Pennsylvania* The increasing use of the polyglycol esters as pharmaceutical vehicles and as an additive in bread to prevent the retrogradation of starch renders important studies of their biochemical behavior The present report gives information on the nutritive value of the esters obtained by combining the fatty acids present in lard with a polyethylene glycol of average molecular weight 400 The plan of the experiment is similar to those described by Lepkovsky, Ouer and Evans (*J Biol Chem* 108:431, 1935) Lard fatty acids were prepared according to the preceding reference Part of the product was esterified with polyethylene glycol 400 to prepare a monoester, while the remainder was re-synthesized to the triglycerides Two groups of twenty four weanling rats each were fed a diet consisting of sucrose 37.5, casein 32.5, salts 1, and cellulose 5 In group I (controls) fat was supplied as 21 parts of the re-synthesized glycerides, while in group II it consisted of 21 parts of the polyglycol ester Purified vitamins were supplied as daily supplements At the conclusion of 17 days feeding the mean weight gain of the polyglycol ester fed group (group II) was 110 gms per rat as opposed to 104 gms in the controls, while the daily food consumption in group II was 10 gms per rat as compared with 8.5 gms in group I Neither of these differences is statistically significant, nor were the growth curves significantly different No evidence of gross pathology was observed in either group at sacrifice

**Comparative effects of d-desoxyephedrine and the isomers of amphetamine on smooth muscle** FRED SHAHLER (by invitation), P L EWING and G A EMERSON *Department of Pharmacology, University of Texas Medical Branch, Galveston, Texas* Quantitative comparisons of the effects of d-desoxyephedrine and d-, l-, and dl amphetamine on intact and isolated smooth muscle of animals are made The property of evincing the phenomenon of tachyphylaxis is confirmed for these compounds On the rat intestine, the inhibitory action of epinephrine is antagonized by these phenalkylamines



and is expressed quantitatively as the p<sub>1</sub>. Other antagonisms are reported which help elucidate the mechanisms of action involved.

**Onset and duration of action of quinidine in the heart after oral administration in man.** S. J. SHAPIRO (by invitation), CONRADO DAVITT (by invitation), JOSEPH G. BENTON (by invitation), ELAINE W. CORLOVE (by invitation), LAWRENCE W. HAYDON (by invitation), WALTER MODELL, and HARRY GOLD. *Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York.* The effect of quinidine sulfate was determined by the change in the speed of the circus movement of the auricle in patients with auricular fibrillation. Each patient received a series of doses, varying from 0.1 to 1.2 gm. at one time, the different doses being given at intervals of several days. After a control electrocardiogram was taken for the measurement of the F-F intervals, additional tracings following the oral dose of the drug were taken at intervals of 2 hours. In a group of 10 patients, the results show that the maximum effect is already present in 2 hours in the vast majority of cases after doses of from 0.1 to 1.2 gm. The effect wears off gradually at speeds varying with the dose. It has completely disappeared in 8 hours after 0.1 gm. and three fourths of the effect disappears in 8 hours after 1.2 gm.

**Methadon derivatives of pharmacological interest.** THEODORE R. SHERROD (by invitation), ROBERT KAISLER (by invitation), J. SANTOS MARTINEZ (by invitation) and CARL C. PFEIFFER. *Department of Pharmacology, University of Illinois College of Medicine, Chicago 12.* From the pharmacological viewpoint it is hoped that at least five types of methadon derivatives may be synthesized: 1) a short acting nondepressant analgesic for use in obstetrics, 2) a long acting orally effective analgesic to obtund the pain of cancer, 3) a potent spasmolytic analgesic compound, 4) a narcotic analgesic with a wide margin of safety for preanesthetic medication, and 5) a potent, persistent analgesic which perhaps, by chemical lobotomy, may permanently lower the pain threshold in causalgic states. With these concepts in mind the following compounds are of interest:

*Therapeutic index*

- |   |     |
|---|-----|
| 1) Methadon HCl   | 2.3 |
| 2) Isomethadon HCl  | 3.1 |
| 3) N, N dimethyl 3, 3-diphenyl 4-imino-2-methylhexylamine HCl         | 4.0 |
| 4) $\gamma$ dimethylamino- $\alpha, \alpha$ diphenyl valeric acid HCl | 5.0 |
| 5) 1 dimethylamino-2-methyl 3, 3 diphenyl 4-acetoxypentane HCl        | 11  |
| 6) 2 dimethylamino-4, 4-diphenyl 5-hexylidenacetylemine HCl           | 12  |
| 7) 2-dimethylamino-4, 4-diphenyl 5-acetoxypentane HCl                 | 14  |
| 8) 2 morpholino-4, 4 diphenyl 5-acetoxypentane HCl                    | 40  |

Compound No. 4 is unique in this series because of an atropine like effect on the intestine of the anesthetized dog. The other derivatives have a spasmogenic effect on the intestine similar to that of methadon. Compounds No. 5 to 8 are of interest because of their high therapeutic indices and long duration of analgesic action. The pharmacology of these compounds will be discussed.

**Blood levels of thiopental (Pentothal) following repeated intravenous administration to the dog.** F. E. SHIDEMAN, A. R. KELLY (by invitation), and B. J. ADAMS (by invitation). *Department of Pharmacology, University of Michigan.* The method of Jailer and Goldbaum was employed to follow blood levels of thiopental in dogs receiving repeated doses of the drug. One group of dogs was used to study the plasma levels of thiopental when moderate doses (10 mg./kg.) were repeated at short intervals (after complete recovery from narcotic action). Under these circumstances, the following evidence suggests that some acute tolerance to the depressant action of this drug is developed. a. The plasma level at which the righting reflexes return is higher with each successive dose even though the actual duration of narcosis progressively increases. b. A significant concentration of the drug is still present in plasma even after complete objective recovery, and this amount increases with each successive dose. Preliminary experiments were done in a second group of dogs to study the plasma levels and decay curve following the single daily administration of a larger dose (20 mg./kg.) of thiopental for 7-9 days. The similarity of the decay curve on the first and last day suggests, but does not prove conclusively, that under these circumstances neither tolerance nor cumulation occurs.

**The effect of hepatic dysfunction in man on the duration of action of thiopental (pentothal).** F. E. SHIDEMAN, A. R. KELLY (by invitation), L. E. LEE (by invitation), V. F. LOWELL (by invitation) and B. J. ADAMS (by invitation). *Department of Pharmacology, University of Michigan and Ypsilanti State Hospital, Ypsilanti, Michigan.* It has been previously demonstrated that the liver is the major organ involved in the detoxication of Pentothal by the rat (Fed. Proc. 6, 344, 1947). The following experiments were performed to determine if such were also the case in man. Bromsulfalein retention and serum proteins were determined in individuals with and without a history of liver disease. Nine patients with a normal bromsulfalein retention and A/G ratio (group A—normal liver function) and six with an abnormally high bromsulfalein retention and an abnormally low A/G ratio (group B—reduced liver function) were selected for study. Each patient received 4 mg./kg. of Pentothal Sodium in a 4 per cent solution intravenously, injected over a period of 30 seconds. Duration of action was determined as the interval

elapsing between the administration of the drug and the time when the individual could stand without support. The significant mean duration of action for group A was 149.4 seconds ( $t = 17.62$ ) and for group B was 488.5 seconds ( $t = 1.72$ ). A significant increase in the duration of action of Pentothal was thus demonstrated in those patients with impaired liver function ( $t$  of the difference of means = 3.32).

**The specificity of the determination of alcohol in biological fluids.** H. WARD SMITH (introduced by G. H. W. LUCAS) *Department of Pharmacology, University of Toronto.* Several high blood values for "apparent" alcohol in cases of diabetic acidosis have been reported in the literature. A reinvestigation was made of the possible values for "apparent" alcohol which appeared in advanced diabetic acidosis in experimental animals and in humans; measurements were made on blood and urine. In the course of this study, it was found necessary to specify completely the conditions for collection and treatment of sample, in order to minimize errors due to contaminants other than ketones. A "specific" method for alcohol was developed which was based on the Widmark desiccation procedure and which used a pretreatment of blood or urine samples by a modification of the Friedmann-Klaas reagents for saliva. The excess dichromate was determined colorimetrically by the use of the chromatediphenylcarbazide complex. When conditions for oxidation were completely specified, a theoretical recovery of alcohol added to blood or urine was obtained, using 0.5 cc. samples with a precision of  $\pm 2\%$  over a wide range of concentrations. It is concluded that conditions can be chosen for the chromic acid oxidation such that the amounts of acetone possible in blood are of no practical significance.

**Method for kymographic recording of CSF pressure, effects of histamine, antistine, PBZ, et al., thereon.** J. SMITH (by invitation), A. CAMEROV (by invitation), B. CRAVER. *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, New Jersey.* The effects of histamine upon canine spinal fluid pressure and the antagonistic actions exerted against them by the two antihistaminics 2-(N-Phenyl-N-benzylamino-methyl)-imidazoline Hydrochloride (Antistine) and N,N-Dimethyl-N'-benzyl-N'-( $\alpha$ -pyridyl)-ethylenediamine Monohydrochloride (Pyribenzamine) were investigated. A method was devised for the continuous kymographic recording of cerebral spinal fluid pressure, since none was available. A blunt 20 gauge needle was inserted into the cisterna magna and the changes in pressure transmitted to a sensitive water manometer. The effects of histamine were quite variable: increases, decreases, but more commonly bi-phasic reactions with an initial fall and a secondary rise. The anti-

histaminics antagonized about equally histamine's action on the CSF pressure and the blood pressure. The effects upon the pressure of a series of standard drugs were also investigated and the conclusion drawn that the effects upon the CSF pressure usually paralleled the effects upon the blood pressure. In general the results suggested that this method was an indirect measure of cerebral blood flow, but this hypothesis could be tested only by concomitant direct measures of the flow. The antihistaminics themselves usually caused a decrease or no change in the CSF pressure so that their clinical use probably would not be contraindicated in conditions in which that pressure was abnormally increased.

**The tissue distribution and toxicity of emetine.** PAUL K. SMITH, ABRAHAM I. GIMBLE (by invitation) and CLARKE DAVISON (by invitation). *From the Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C.* Methods were investigated for the determination of the distribution of emetine in animal tissues. Results, using the methyl orange technique described by Brodie and Udenfriend (*J. Biol. Chem.* 158, 705-14, (1950)) and a method utilizing the ultra-violet absorption bands, were in satisfactory agreement. Two hours after intraperitoneal injection of the drug in rats, the concentrations were highest in the liver with appreciable amounts in the lung, kidney and spleen. The concentrations in muscle, brain and blood were very low. In spite of the high cardiac toxicity of this substance, the concentrations in the heart were also low. After 48 hours, the concentrations in the liver, lung and kidney were approximately half the concentrations found at the end of two hours. Samples of the whole mouse were analyzed at intervals up to 35 days after intraperitoneal administration of the drug. The amount of emetine recovered decreased slowly until approximately the eighth day and remained approximately constant for at least 35 days with doses varying from 40 to 80 mgm per kgm. The amount recovered after eight days was fairly constant, that is, there had apparently been a greater excretion or destruction of the drug after high doses than after low doses. The intraperitoneal  $LD_{50}$  in rats was approximately 17 mgm per kgm and in mice 62 mgm per kgm over a seven day period.

**The distribution and excretion of antimony following administration of antimony potassium tartrate and neostibosan.** RALPH G. SMITH, JAMES Y. P. CHEN (by invitation) and MARGUERITE MAGLE (by invitation). *Department of Pharmacology, Tulane University School of Medicine, New Orleans.* Antimony potassium tartrate (10 mg/kg) and Neostibosan (80 mg/kg) representing tervalent and quinquevalent antimony compounds were injected intraperitoneally in single doses into hamsters. Series of animals were sacrificed at 1, 6,

12, 24 or 48 hours after injection. Blood, heart, lungs, spleen, liver, muscle, bone, kidney, bile, gastrointestinal tract and contents, urine and feces were analyzed for antimony by the colorimetric method of March. With antimony potassium tartrate a decrease in antimony concentration in tissues and blood usually occurred after 6 to 12 hours but the content 48 hours after administration was at least 50 per cent of that at the one hour period, with the exception of kidney, gastrointestinal tract and bile which showed still lower values. The average concentrations in heart, spleen, liver, bone and gastrointestinal tract were higher at the 6 or 12 hour periods than 1 hour after injection but the differences involved were not statistically significant. With Neostibosan the decrease in antimony concentration over the 48 hour period was more rapid falling to from 20 to 50 per cent of the one hour values. Kidney and liver on the contrary showed maintained or increasing concentrations throughout. In comparison with other tissues, high concentrations of antimony occurred in liver, bile and somewhat in spleen after antimony potassium tartrate and in spleen, kidney, liver and bile after Neostibosan. In accord with previous reports more antimony was excreted by the bowel than by the kidney after antimony potassium tartrate, the reverse occurring after Neostibosan.

**Initial studies of the inhalation toxicity of beryllium sulfate.** GEORGE F. SIRACI (by invitation), ALTON G. PETTENCILL (by invitation), and HERBERT C. STOKINGER (by invitation). Introduced by HARVEY B. HALE. Data from 56 animals representing 6 species were collected to supply information on the toxic effects caused by the inhalation of beryllium sulfate tetrahydrate at a concentration approximating 90 mg of the salt per cubic meter of air. The animals were exposed 6 hours daily for a two week period in a small inhalation exposure chamber. **Mortality**, for all species, was 43% of the exposed animals or 20 of 20 mice, 2 of 10 rats, and 2 of 7 hamsters. No deaths occurred among the 2 dogs, 3 rabbits and 14 guinea pigs. **Weight response** data showed that all species save the guinea pig were adversely affected by the exposure. The rat and hamster showed greatest weight depression, losing 11% of their original weight. **Clinical chemical** values indicated renal impairment and some subsequent regeneration in the rabbits. Much less serious kidney damage and hepatic injury in dogs was indicated from biochemical tests performed on both blood and urine. **Hematologic** results showed definite upward trends in the leukocytic count during the second week of exposure. **External symptoms** differed in extent in each species. Thus, ocular opacity developed in the dog, mouse and guinea pig whereas the rat developed rales, other species were unaffected externally. The **histologic** findings showed the effect

of the absorption of  $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$  through the respiratory tract of the mouse, rabbit and rat to be pulmonary edema. Hepatic injury was observed in mice dying during the first 9 days, but not there after. Renal changes were found in the rabbit and rat with some regeneration of the tubular epithelium in the former species. Marked species variation, but good conformity within each species, was observed in the response to the inhalation of  $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$ . The sulfate ion was felt to contribute significantly to the toxicity of the beryllium salt.

**The blocking action of magnesium ion on sympathetic ganglia.** JOHN B. STANBURY (introduced by OTTO KRAYER). *Department of Pharmacology, Harvard Medical School.* If magnesium ion is injected intravenously, or into the arterial blood supply of the ganglia in cats during preganglionic stimulation of the superior cervical ganglion, the stellate ganglion, or the inferior mesenteric ganglion, a brief blockade of impulse transmission occurs. This blockade is located at the ganglion, since the injection has no influence on conduction in the pre- and postganglionic fibers. Magnesium ion has a relaxing action on the mediating membrane independent of its effect on the superior cervical ganglion, and a decelerating effect on the heart independent of its action on the stellate ganglion. Magnesium ion given intravenously lowers the blood pressure in the intact cat as well as when sympathetic tone has been eliminated by pithing or by giving a constant infusion of epinephrine. This suggests that the blood pressure lowering action of magnesium ion is not wholly attributable to its effect on the sympathetic ganglia.

**Potentiality of the effect of antihistaminic agents by iron compounds.** GEORGE W. STAVRAKY. *Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada.* Noting that  $\text{Ca}^{++}$  is necessary for blood clotting, neutral salts and the action of heparin, and iron containing substances play an important role in intracellular respiration, it was decided to investigate metallic compounds as possible activators of antihistaminic agents. While Ca and K were ineffectual, Ferrous Sulphate, in decerebrated vagotomized cats, increased the effectiveness of small doses of Antistine in counteracting blood pressure changes induced by histamine. Experiments on 8 patients with seasonal hay fever and asthma due to ragweed showed that administration of Ca or K with antihistaminic drugs was again ineffectual whereas Ferrous Sulphate by mouth in daily doses of 20-45 grains added to Pyribenzamine or Antistine was most effective in alleviating allergic manifestations. Iron also seemed to relieve the lassitude and drowsiness induced by antihistaminic agents, this latter action made it possible to increase the doses of Pyribenzamine and Antistine with further benefit to the patients (50-100 milligrams Pyribenz

amine sometimes combined with as much as 200 milligrams Antistine, and 0.5-0.6 grams Ferrous Sulphate were given 1-5 times a day without ill effects. As a precaution, iron was given with calcium which is known to reduce the toxicity of iron. One patient, in spite of taking 600 milligrams of Antistine daily, developed asthma which was not materially helped by intramuscular injections of epinephrine in oil. When put on antihistamine therapy he was relieved from asthma within 45 minutes and continued in relative comfort throughout the remainder of the hay-fever season in the face of an increasing pollen count.

**Adrenergic blocking properties of certain halogenated ethylamine derivatives.** CHAS A. STONE (by invitation), PAUL ACHINBACH (by invitation) and EARL R. LOEW, *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago*. Experiments were made to study the adrenergic blocking action of N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr and  $\beta$ -2-biphenyloxyethyl-butyl  $\beta$ -chloroethylamine HCl, which have been shown previously to have the ability to block certain effects of epinephrine as well as histamine (Fed. Proc., 6: 304, 1947). Adrenergic activity was induced in dogs lightly anesthetized with pentobarbital sodium by (1) occlusion of the common carotids, (2) production of anoxia ( $N_2$  inhalation) and (3) injection of small doses of nicotine; the response in each case was manifested as a rise in blood pressure. The effect of the compounds on the pressor response to injections of epinephrine was also determined. In control experiments made in animals receiving no treatment the above responses remained essentially unaltered for three or four hours. The intravenous doses of N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr and  $\beta$ -2-biphenyloxyethyl-butyl  $\beta$ -chloroethylamine HCl were 1 mgm./kgm. and 5 mgm./kgm., respectively. In both atropinized and unatropinized animals both compounds reversed the pressor effects of epinephrine and blocked or reversed the responses to nicotine and anoxia. The pressor responses to carotid occlusion were diminished to a significant degree but were not blocked, possibly because liberation of epinephrine from the adrenal medulla is not concerned in this reaction to the extent that it is in responses to nicotine and anoxia. This evidence, in conjunction with results of previous studies, indicates that N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine HBr is a more potent adrenergic blocking compound than  $\beta$ -2-biphenyloxyethyl-butyl  $\beta$ -chloroethylamine HCl and that the former compound is also highly effective in antagonizing histamine.

**Effects of alterations in body temperature on properties of convulsive seizures in rats.** EWARL A. SWINYARD (by invitation) and JAMES E. P. TOMAN, *Departments of Pharmacology, Pharmacy,*

*and Physiology, University of Utah, Salt Lake City, Utah*. Threshold and pattern of electrically and chemically induced convulsions were studied in rats whose body temperature was experimentally varied by environmental heating or cooling. **Seizure threshold.** Electroshock threshold (60 cycle A.C., 0.2 sec. duration) for minimal seizures was decreased by lowering and increased by elevating body temperature. The  $Q_{10}$  was 1.5 at 25°-35°C and 1.8 at 35°-45°C. The results suggest an absolute increase in electrical excitability at reduced temperatures, modified by an increase in time constant of excitation. The thresholds for Metrazol and Picrotoxin seizures were also reduced by lowering body temperature ( $Q_{10}$  approximately 2). Extremes in body temperature (below 25°C and above 43°C) were convulsant to some animals. **Recovery.** The effect of body temperature on recovery rate of minimal seizure threshold following maximal seizures was studied. The time for recovery to 150% of threshold was approximately doubled by a 10°C reduction in body temperature ( $Q_{10}$  approximately 2). **Seizure Pattern.** The  $\log_{10}$  of maximal seizure duration varied linearly with the reciprocal of absolute temperature, giving a  $Q_{10}$  of 2.8. The tonic extensor component was decreased in relative duration either by lowering or elevating body temperature, and was abolished below 27° and above 42°C.

**Some metabolic products of para-aminobenzoic acid.** CHAS. WHITE TABOR (by invitation), JUDITH BARRY (by invitation) and PAUL K. SMITH, *From the Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C.* In continuation of our studies on the fate of para-aminobenzoic acid, glycuronate excretion was determined following the oral administration of para-aminobenzoic acid or the sodium salt, using the carbazole method recently described by Dische (J. Biol. Chem. 167, 189-198 (1947)). In patients given single six gram doses of the drug or smaller doses every two hours, relatively large quantities (up to 2.7 gm.) of apparent glycuronate were present in twenty-four hour urine specimens. The changes in the excretion rate of this substance were roughly parallel to the change in the excretion rate of free amine. Using the countercurrent distribution method of Crug (J. Biol. Chem. 155, 519-54 (1944)) to supplement other methods, it was determined that the metabolic products of para-aminobenzoic acid probably include the free acid, para-aminohippuric acid, the acetylated forms of each, and a glycuronate derivative of a free amine. These studies suggest that the metabolism of this drug is similar to that of benzoic acid (Snapper, Am. J. Med., II, 327-333 (1947)) and some of the sulfonamides.

**Urinary excretion of histamine following oral administration, employing an improved colorimetric method.** HIRSHL TAVOR and SANFORD M.

ROSENTHAL *Division of Physiology, National Institute of Health, Bethesda, Maryland* A colorimetric method for histamine, based upon a diazo reaction with 4 nitro aniline, has been developed. By the use of a solvent (methyl isobutyl ketone) the color is extracted and then brought to a pH of 7.7 by shaking with barbital buffer. This procedure is sensitive to approximately 0.5 gamma of histamine base, and results upon animal tissues indicate a satisfactory degree of specificity. Anrep has shown that histamine can occur in the urine in free and conjugated form. A corresponding study of urinary histamine following oral administration was carried out in several species of animals. Up to 35 per cent of the amount given could be recovered. There was considerable species variation in the total excretion, and in the amount conjugated.

**Some effects of ouabain and/or calcium in mice**  
ROBERT TARAIL and W. LANE WILLIAMS (Introduced by R. N. BIERER) *Departments of Anatomy and Medicine, University of Minnesota* Mice (av wt 25 gms.) were daily injected subcutaneously or intraperitoneally with (doses expressed in mg. per 100 gms. body weight) (a) ouabain, 0.5 to 2 mg., for 1 to 36 days, or (b) calcium chloride, 33 to 50 mg., or calcium gluconate, 100 to 200 mg., for 13 to 19 days, or (c) with combinations of ouabain and calcium in amounts representative of the range of doses listed in "a" and "b." Total daily amounts of ouabain greater than 0.25 mg. were injected in divided doses over a period of 1 hour since larger single doses often resulted in immediate death. Most of the treatments with one of the compounds and all of the combined treatments produced small focal areas of myocardial necrosis. Mice receiving ouabain (alone) showed in addition to myocardial lesions some edema, venous and capillary congestion in kidneys, liver, and lungs. In addition to these changes treatment with calcium or combinations of calcium and ouabain produced necrosis and hemorrhage in the livers and kidneys. An increased dual toxicity of combinations of ouabain and calcium was suggested by reduction in life expectancy in relation to that observed in mice on single treatment with either calcium or ouabain. However, any increase in the toxicity and lethality of combinations of ouabain and calcium could not be quantitatively correlated with increased total tissue damage (heart, liver, kidneys, and lungs) and such doses demonstrated no clear synergistic or cumulative effects in producing morphological myocardial injury.

**The pharmacology of organic thiocyanates**  
SILAB A. ABDEL TAWAB (introduced by JOHN C. KRANTZ, JR.) *Department of Pharmacology, Univ. of Maryland, School of Medicine, Baltimore* The study of some organic thiocyanates revealed the depressor action of these compounds especially with p-thiocyanophenol. Further study of this sub-

stance showed that it diminishes the oxygen consumption in white albino rats together with pathological changes in the thyroid gland analogous to those of colloid goiter. The insolubility in water and the high toxicity of this compound has led us to suggest the synthesis of thiocyanobenzoic acid derivatives, the sodium salts of which are soluble in water and the benzoic derivative is comparatively non-toxic. Sodium p-sulfocyanobenzoate is found to have a prolonged depressor action with slow return to normal and marked respiratory stimulation—an action which is believed to be central in origin. Comparative study with sodium thiocyanate showed that the sulfocyanate radical is about 80 times more potent than the radical in polar combination with an alkali cation.

**Factors affecting the use of lung thromboplastin in the determination of prothrombin time**  
BERNARD G. H. THOMAS (by invitation), JACQUELINE SIFFERS (by invitation), and CHARLES R. LINCOLN *Pharmacological Development Division, E. R. Squibb & Sons, New Brunswick, New Jersey* Dried thromboplastin from rabbit lung was found to be more stable at room temperature and more potent, and to give better correlation of clotting time with various prothrombin levels in undiluted and saline diluted plasma than thromboplastin from rabbit brain. Lung thromboplastin showed greater activity when extracted with an aqueous solution containing both sodium and calcium chloride rather than with one containing only sodium chloride. The addition of 0.4 per cent phenol to the extracting fluid increased in most cases the activity of the extract, and preserved the thromboplastic activity for approximately 24 hours when the extract was stored at room temperature. Factors lengthening the prothrombin time were found to be the removal of part of the suspended thromboplastin by centrifugation of the extract, the addition of inadequate or excessive amounts of calcium chloride in the test, the use of more than the required amount of oxalate in the collection of blood samples, the presence of clots in the blood sample to be tested, and undue delay in performance of the test. In order to evaluate prothrombin times properly, the tests should be made with a standardized thromboplastin under carefully controlled conditions.

**Effect of adrenolytic agents on the response to pressor substances in the domestic fowl**  
RONALD M. THOMPSON (by invitation) and JULIUS M. COON *Dept. of Pharmacology, University of Chicago* It has been shown that dibenamine HCl causes a reversal of the pressor response to epinephrine in mammals. Intravenous dose of 20 mg./kg. of dibenamine HCl neither reversed nor depressed the pressor responses to small or large doses of epinephrine in the domestic fowl. Dibenamine also did not affect appreciably the pressor responses (in amounts equivalent in pressor effect to 0.1 cc. of

epinephrine, 1:100,000) to benzedrine, cobefrin, ephedrine, neosynephrine, synephrine, tyramine or nicotine in the fowl. However, dibenamine-HCl markedly diminished the pressor response to the equivalent amount of Vumon (2,1 dimethoxy- $\alpha$ -phenyl beta methylamino ethanol Prisol and dihydrocrotamine, unlike dibenamine-HCl, diminished (though did not reverse) the pressor response to epinephrine. This is interpreted as indicating a difference in the mechanism of action of these two adrenergic drugs from that of dibenamine-HCl. The domestic fowl was markedly resistant to the toxic effects of epinephrine. As much as 30 mg/kg of epinephrine (1:100) injected rapidly intravenously did not cause the immediate death that occurs in mammals given much smaller amounts. However, doses upward from 2.5 mg/kg of epinephrine caused immediate prostration, followed in about 10 minutes by extreme difficulty in the expiratory phase of respiration, and death in 10 to 120 minutes. Death appeared to be the result of increasing respiratory difficulties. An intravenous dose of 10 mg/kg of epinephrine one hour after 20 mg/kg of intravenous dibenamine HCl caused no prostration, only slight respiratory difficulty, and full recovery in the majority of chickens. Thus, dibenamine-HCl protected the domestic fowl against the toxic effects of large doses of epinephrine although it did not affect the pressor response to epinephrine.

**Effect of convulsant and anticonvulsant agents on acetylcholine metabolism and effector organs' acetylcholine sensitivity.** CLARA FORDA and HAROLD G. WOLFE. *New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York, N. Y.* It has been reported that acetylcholine applied locally to the motor areas of the cerebral cortex or administered parenterally in animals induces changes in the electroencephalogram resembling those observed during 'grand mal' attacks in man. In the following it was ascertained whether or not the convulsion inducing agents (pentamethylene tetrazol, picrotoxin, strychnine, morphine, camphor, scilliroside, digitoxin) (a) increase the synthesis of acetylcholine (increased activity of choline acetylase), (b) decrease the hydrolysis of acetylcholine (decreased activity of cholinesterase), and (c) increase the sensitivity of effector organs to acetylcholine. All of these measures, together or separately, may induce a temporary accumulation of acetylcholine. It was also ascertained whether or not the anticonvulsant agents (hydantoin, methyl-phenyl ethyl hydantoin, diphenylhydantoinate sodium, trimethadione, sodium bromide, phenobarbital, barbital sodium, pentobarbital sodium, diallylmalonylurea, iso amyl-ethyl barbiturate sodium) (a) decrease acetylcholine synthesis, (b) increase the hydrolysis of

acetylcholine, and (c) decrease the sensitivity of effector cells to acetylcholine. These measures, together or separately, may induce a temporary decrease of acetylcholine. The results show that most of the convulsion inducing agents cause an accumulation of acetylcholine and most of the anticonvulsant agents cause a decrease in the concentration of acetylcholine. There are, however, convulsion inducing and anticonvulsant agents that do not modify acetylcholine metabolism in the above demonstrated manner. It seems that accumulation of acetylcholine per se is not the primary factor in the induction of convulsive seizures nor is lack of acetylcholine the primary factor in prevention of convulsive seizures.

**The quantitative estimation of theophylline in blood.** EDWARD B. TRUITT, JR. (introduced by JOHN C. KRANZ, JR.) *Department of Pharmacology, Univ. of Maryland, School of Medicine, Baltimore.* A colorimetric method for the quantitative estimation of theophylline in blood has been developed. The method is based on the formation of a red color through the coupling of a diazo compound, the diazotized 5-amino-2-benzoylamino-1,4-dithoxybenzene with an alkaline oxidation product of theophylline. The reaction produces a comparatively stable red color that will detect theophylline in blood levels of 0.1 mgm % or more. The method has been applied to patients undergoing theophylline therapy.

**The spreading of Evan's blue injected intradermally.** RUTH UBER (by invitation), PATRICIA COLLINS COWDERY (by invitation) and GEORGE E. FARRAR, JR. *Dept. of Medicine, Temple Univ. School of Medicine, Philadelphia, Pa.* Following the intradermal injection of 0.1 cc of 0.24% Evan's blue in aqueous 0.9% sodium chloride solution, the longest and shortest diameters of the blue area were measured. The spreading index was calculated, — square of the median radius at 3 and 24 hours divided by this figure for the initial wheal. The degree of spreading varied greatly among both healthy and diseased persons but was repeatedly similar in an individual. The average spreading index in a group of 30 healthy, young adults (15 men, 15 women) was 1.5 at 3 hours, with a range of 1.5 to 10.7, and 10 at 24 hours, 1.5 to 23.5. The degree of spreading at 3 hours seemed to be little affected by factors such as exercise and weather which may alter the reading at 24 hours considerably. Patients with rheumatic fever showed no gross differences, 5 cases in the active phase had an average index of 3.1 at 3 hours, 5 inactive cases averaged 4.5. Fifteen cases of active rheumatoid arthritis had an average index of 11.2 at 24 hours with a range of 5.4 to 29.6. Ten patients with far advanced pulmonary tuberculosis had an average of 6.6 and 10 minimal cases 5.1 at 3 hours. Pregnant patients before and after delivery and cases of pericarditis nodosa, disseminated lupus

erythematosis and osteoarthritis have been studied. No correlation has been found between spreading index, erythrocyte sedimentation rate and the inhibiting effect of the blood serum on the action of hyaluronidase derived from hemolytic streptococci of group A.

**The estimation of alpha and beta estradiols and of estrone and equilin in binary mixtures.** ERNEST J. UMBERGER and JACK M. CURTIS (introduced by ARNOLD J. LEHMAN). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.* The behavior of several natural estrogens toward various concentrations of sulfuric acid has been studied. These studies have provided a method for the identification and quantitative estimation of the estrogens after their partial separation by chromatography. Beta estradiol may be determined by heating with a solution of 30% sulfuric acid and 20% n-butyl alcohol in water on a boiling water bath for 6 minutes and reading the color at 524 m $\mu$ . Total estradiols are determined by heating with 90% sulfuric acid for 15 minutes and reading at 444 m $\mu$ . Alpha estradiol is determined by difference. Estrone and equilin may be determined simultaneously by heating with 90% sulfuric acid for 5 minutes and reading at 450 and 480 m $\mu$ . The proportion of estrone or equilin can then be calculated by simultaneous equations or by applying the formula of Knudson, Meloche, and Juday (in Ed., *Ind Eng Chem* 12, 715, 1940).

**Efficacy of atropine in children.** K. R. UNNA, K. GLASER (by invitation), E. LITTON (by invitation), and P. PATTERSON (by invitation). *Departments of Pharmacology and Pediatrics, University of Illinois College of Medicine, Chicago 12, Illinois.* Effects of graded doses of atropine following oral and subcutaneous administration were determined in children aged 1 month to 12 years. The suppression of the stimulation of salivary flow by chewing gum over a specified 5 minute period was chosen as the criterion for threshold effects in older children. The threshold doses determined by the chewing test were identical with those suppressing the flow of saliva elicited by small doses of methacholine injected subcutaneously. In children below the age of 3 years, minimum effective doses of atropine were estimated by the saliva flow after methacholine. Although appreciable individual variations, especially on oral administration, were sometimes encountered, the average threshold doses in the various age groups (1-12 months, 1-3 years, 3-6 years, and 6-12 years) were found to lie in a narrow range from 15 to 23 micrograms after oral doses and from 4 to 6 micrograms after subcutaneous doses of atropine per kgm. body weight. Slightly larger doses often caused flushing and febrile reactions in small children. Still larger doses were required to raise the pulse rate and to dilate

the pupils. The implication of these studies on the concept of physiological "vagotonia" in young children will be discussed.

**A comparison of curare alkaloids.** E. F. VAN MANNEN (introduced by OTTO KRAHLER). *Department of Pharmacology, Harvard Medical School.* The preparations studied included d-tubocurarine, c. toxiferine II and an extract of the bark of *Strychnos Toxicaria*. These preparations decrease the sensitivity of the isolated rectus abdominis of the frog to acetylcholine. The shape of the dose response curve suggests a competitive reaction between acetylcholine and the curare alkaloids. The relative activity of the alkaloids on the rectus abdominis does not correspond to their relative lethal dose in mice, injected intravenously. Of the three alkaloids named the relative activity in the rectus was, respectively, 1, 2.5 and 0.3. The corresponding LD 50's in mice were 1, 0.62, and 0.044 (for the extract, the dry weight was used in calculating these results, 1 gram of extract, dry weight, corresponds to 3 grams of bark). In rats anesthetized with urethane, twitches from the Achilles tendon on maximal stimulation of the sciatic nerve were studied after intravenous injections of the alkaloids. From the time course and extent of the neuromuscular block deductions can be made concerning rates of binding of the alkaloids to muscles and rates of elimination of the alkaloids from the body.

**The rabbit "head-drop" method for the biological assay of curare and its alkaloids.** ROGER F. VARNLEY (by invitation), CHARLES R. LINEGAR and HORACE A. HOLADAY (by invitation). *Biological and Chemical Laboratories, E. R. Squibb & Sons, New Brunswick, New Jersey.* In the early development of standardized curare, the classical methods of assay failed to serve as a reliable index of potency of various curare preparations in humans. In this respect the rabbit "head drop" method which employs muscular relaxation in an intact mammal as the criterion of curare activity has proven best. To reach "head drop" a rabbit, tied belly down, is given an interrupted intravenous injection of a solution containing 2 units per ml.  $\pm$  10 per cent via a microburette at the rate of 0.1 ml. every 15 seconds until muscles supporting the head become sufficiently relaxed to prevent its being raised when the back is stimulated. The end point is sharp and reproducible. The "head drop" dose in different rabbits even of the same weight varies widely, therefore it is of utmost importance to compare the potency of an unknown curare with that of a standard in the same animal. Since, following preliminary curarizations, the daily "head-drop" dose requirement of each rabbit is quite constant, the assay is applied as a two day "cross over" test. In 550 determinations of the ratio of activity of standard versus unknown in randomly selected rabbits,

the standard deviation of a single ratio was calculated and on this basis the standard error for the mean of ten ratios was 3.9 per cent. The curare unit originally defined as the activity of 1 mg. of a desiccated curare powder was later shown to be equivalent to about 0.15 mg. of the "pentahydrate" of *d*-tubocurarine chloride.

**Comparative studies of the toxicity and physiological action of chlorinated methanes with reference to their physical and chemical characteristics.** W. F. VON OTTINGEN, C. C. POWELL (by invitation), N. E. SHARPLESS (by invitation) and L. J. PECORA (by invitation). *From the Laboratory of Physical Biology, National Institute of Health, Bethesda, Md.* The LD<sub>50</sub> for mice by inhalation for 7 hours of mono-, di-, tri-, and tetrachloromethane was determined, and the effect of these chlorinated methanes on the circulatory and respiratory apparatus, the nervous system, and their concentration in heart, liver, brain, and blood with exposure to concentrations of 15000 p.p.m. was studied in dogs. The reaction rate constant and the partition coefficient in a system oleic alcohol/water was determined. It was shown that monochloromethane is readily decomposed and that this is also reflected in its physiological action and distribution in the organs studied. Di-, tri- and tetrachloromethane lowers increasingly the blood pressure in the order given, only the two latter cause direct cardiac depression under the conditions studied. Whereas monochloromethane causes first a marked stimulation of the respiration and only a depression after a latent period of several hours, di-, tri-, and tetrachloromethane cause a depression, increasing in the order given. Whereas the depressant effect of monochloromethane on the nervous system is very moderate, this increases in intensity from di-, over tetra-, to trichloromethane. The organ distribution of the chlorinated methanes shown that in the case of tetrachloromethane there is a distinct and with trichloromethane a less marked accumulation in the central nervous system. The parallelism between the physiological behavior and certain physical-chemical characteristics is discussed.

**The paralytic and lethal action of myanesin, pentobarbital and combinations of these agents in mice.** H. A. WALKER (by invitation), ARTHUR P. RICHARDSON, P. LOEB (by invitation) and J. PEROG (by invitation). *Department of Pharmacology, Emory University School of Medicine, Emory University, Georgia and Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, New Jersey.* We have been unable to confirm the conclusions reached by Berger and Bradley (*Brit. J. Pharmacol.* 1, 265 (1946)) that the combined depressant effect of myanesin and a barbiturate is greater than might be expected from the individual activities of each agent. Loss of righting reflexes was taken as a criterion of paralysis. White

mice of 18-22 grams were injected intraperitoneally with varying doses of myanesin and sodium pentobarbital, and various combinations of these two agents. Any animal unable to right himself within 15 seconds after being placed on his back was considered paralyzed. All animals were held for 24 hours and deaths noted for this period of time. The PD<sub>50</sub> for myanesin alone was found to be 182 mgms per kilo, and for sodium pentobarbital 43 mgms per kilo. In mice injected with 90 mgms per kilo of myanesin (50% of PD<sub>50</sub>), 19.5 mgms per kilo of pentobarbital (45.5% of PD<sub>50</sub>) were required to produce paralysis. This result is within 5% of the expected additive effect. In other series of mice receiving from 12.5 to 75% of the PD<sub>50</sub> of myanesin plus the barbiturate similar results were obtained. The lethal effects of combinations of these two agents were also found to be purely additive.

**Comparative increase in ventricular contractile force produced by several cardiac glycosides.** R. P. WALTON, M. F. PATTON (by invitation), H. P. JONES (by invitation) and J. S. LEARY (by invitation). *Dept. of Pharmacology, Medical College of South Carolina, Charleston.* Five cardiac glycosides and tincture of digitalis were compared at dose levels ranging from 0.25 to 1.3 cat units per kgm. Determinations of ventricular contractile force (isometric systolic tension of a section of the right ventricle in open-chest dog preparations) were made according to the method described in J. P. D. T., 89, 26, 1917. The glycosides used were ouabain, thevetin, scillaren, strophanthidin acetate and lanatoside C (Cedilanid). Total doses were administered during a period of 30 minutes at uniform rates of infusion. With doses of 0.25 cat units per kgm, maximum effects were obtained in an average of 42 minutes from the beginning of the infusion. Increasing doses progressively produced shorter intervals before development of maximal effects. With doses of 1.3 cat units per kgm, maximum effects were obtained in an average of 22 minutes. Effects developed most rapidly with thevetin and most slowly with tincture of digitalis and with lanatoside C. Comparison of the increases in contractile force produced by the individual members of this series supports the conception of their basic similarity. Possible deviations, which are being further examined, indicate, in the lower dosage range, a greater than average effect with thevetin and a less than average effect with tincture of digitalis. Based on data obtained from 15 to 20 experiments with each glycoside, the average maximal increases in contractile force (expressed as percentage increase over that of the control period) were as follows: 0.25 cat units, 31%, 0.50 cat units, 46%, 0.75 cat units, 60%, 1.0 cat units, 93%, 1.3 cat units, 100%.

**Hypnotic properties and toxicity of 2-ethyl-3-propylglycidamide.** MARSHALL R. WARREN (by



invitation), CHARLES R THOMPSON (by invitation), and HAROLD W WERNER *Pharmacology Dept., Research Labs., The Wm S Merrell Co., Cincinnati, O* 2 Ethyl 3 propylglycidamide has a central depressant action in experimental animals generally resembling that of shortacting barbiturates grossly, and it also antagonizes metrazol-induced convulsions. Intravenous studies in rabbits demonstrate that the acute  $LD_{50}$ s for 2 ethyl-3-propylglycidamide, pentobarbituric acid, and ethylisoamylbarbituric acid are  $> 360, 34,$  and  $55$  mgm /kgm respectively, and minimal hypnotic doses (smallest doses which caused 50 per cent or somewhat more of the animals receiving them to be on their sides with heads down) are  $70, 10,$  and  $13$  mgm /kgm. All three compounds have similar durations of action since the intravenous administration of two minimal hypnotic doses causes an average narcosis of 1.4 to 1.6 hours. Oral studies in rats show that the acute  $LD_{50}$ s for 2 ethyl-3-propylglycidamide, pentobarbituric acid, and  $\alpha$ -monobromo-isovalerylurea (Bromural) are  $125, 0.125,$  and  $1.0$  gm /kgm respectively, and minimal hypnotic doses are  $0.50, 0.063,$  and  $0.50$  gm /kgm. All three substances are similar in duration of action following oral administration in rats since one minimal hypnotic dose produces a median narcosis of 3.3 to 4.5 hours. Repeated daily administration of 2 ethyl-3-propylglycidamide, in doses equal to one tenth and two-fifths of the oral minimal hypnotic dose for rats, to rats, dogs, and monkeys for periods of four to eight weeks gave no evidence of toxicity or cumulative action. Hematologic studies on the experimental animals revealed no alteration of the blood picture from that of the control animals. Microscopic examination of tissue sections showed no pathologic changes which could be attributed to drug administration.

**Effect of some central nervous system stimulants and depressants on the activity of succinic dehydrogenase.** D T WATTS (introduced by C L GEMMILL) *Department of Pharmacology, University of Virginia, Medical School, Charlottesville, Virginia*. The effect of selected central nervous system drugs on succinic dehydrogenase activity in vitro has been determined, using the Thunberg technique. The rate of methylene blue reduction by the enzyme preparation from washed frog muscle with sodium succinate as substrate was followed with a photoelectric colorimeter or by determining an end point visually. Barbitol, pentobarbitol sodium, ethyl urethane, chloral hydrate and sodium bromide gave no inhibition at final concentrations of  $1.2 \times 10^{-3}$  to  $4.0 \times 10^{-2}$  M. Caffeine produced inhibition at concentrations as low as  $1.3 \times 10^{-3}$  M and gave complete inhibition at  $2.1 \times 10^{-2}$  M. Similar inhibition was observed with theophylline. A technique has been developed for passing volatile anesthetics into evacuated Thun-

berg tubes either in the gaseous state or by allowing the liquid to distill into the tube. Chloroform inhibits this dehydrogenase. This loss of activity can be partially restored by boiling off the chloroform by additional evacuation.

**The fate of p-aminosalicylic acid in the animal body.** E LEONG WAI, ROWENA WEISS (by invitation), DONALD L HOWIE (by invitation) and PAUL K SMITH *From the Department of Pharmacology, The George Washington University School of Medicine, Washington, D C*. The absorption, distribution and excretion of p-aminosalicylic acid (PAS) was investigated in the rat, dog and man by determining free amine, conjugated amine and free phenol. There is little or no storage of PASA in rats after intravenous or oral dosage (200 mgm per kgm). Within four hours, the compound is found chiefly in the urine, with a small fraction in the gastrointestinal tract. By far the highest tissue concentrations are attained in the kidney, then in the lung and liver, with the latter yielding appreciable values for conjugated amine. In dogs, about ninety per cent of an intravenous 100 mgm per kgm dose of PAS can be recovered in the urine as free amine or free phenol after eight hours. The rate of fall of plasma levels closely parallels the rate of appearance in the urine. In humans, after an oral four gram dose, plasma levels rise rapidly to a maximum of approximately 10 mgm per cent and then fall rapidly, with over ninety per cent of the total dosage being recovered as total amine or free phenol. Even after five or six single 2.5 gram doses at six hour intervals, over eighty per cent of the total amount given can be accounted for within six hours after the last dose, with about fifty to sixty per cent being present as conjugated amine. On comparing the partition coefficients of urinary products and of added PASA in ether and acetate buffer (pH 3.4), it was found that the free amine in urine is present in a more water soluble form.

**Metabolism of rat heart slices.** J LEYDEN WEBB (by invitation), PAUL R SAUNDERS (by invitation) and CLINTON H THIENES *Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles, California*. The respiration of slices of rat ventricular myocardium was studied in the presence of various substrates and inhibitors. Substrates fall roughly into four classes according to the ability of the heart to utilize them: increase respiration over 200 per cent (succinate), from 50-100 per cent (citrate, oxaloacetate), from 10-30 per cent (pyruvate, lactate, malate, hydroxybutyrate) and no effect (ketoglutarate, glucose, glucose-1-phosphate, hexose diphosphate). Glucose has no immediate effect but after two to three hours the respiration begins to rise until it is higher than initially, thus glucose maintains respiration but requires an appreciable time to be utilized. Malonate, at a con-

centration (0.02M) completely inhibiting succinate oxidation, inhibits the endogenous respiration of heart slices 30 per cent. With concentrations of malonate between 0.002M and 0.02M the respiration rises from its originally depressed value for 90 minutes and then falls off more slowly than control, at 90 minutes the respiration may be higher than control. A type of respiration that is malonate-insensitive seems to increase during this period. Preliminary evidence indicates the malonate is responsible for this. This malonate "hump" occurs only in the presence of calcium. Fluoride inhibition of cardiac respiration increases with fluoride concentration up to 0.01M but then decreases to a minimum when fluoride is 0.05M and then increases again as fluoride concentration raised. Iodoacetate and phlorizin are very potent inhibitors of cardiac respiration while pyrophosphate is a relatively weak inhibitor.

**The influence of N-(2-bromoethyl)-N-ethyl-1-naphthalene-methylamine on the vasopressor response of a series of amines.** J. A. WELLS and DAVID P. RALL (by invitation) (*From the Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois*). Reversal of the pressor action of epinephrine characteristically follows the administration of potent sympatholytic drugs. Occasionally this same phenomenon has been observed with other pressor amines, but some difference of opinion exists as to which compounds are reversed and some are claimed never to be reversed. The compound, N-(2-bromoethyl)-N-ethyl-1-naphthalene-methylamine (SY 28), recently synthesized and supplied to us by Doctors Rieveschl, Fleming and Coleman, has been shown by Dr. Earl Loew to be a potent sympatholytic drug. We have been interested in knowing which of the pressor amines are blocked or reversed by this drug. Following the slow intravenous administration of 1-5 mg/kg of this compound to nembutal anesthetized dogs, cobefrine, adrenalone and tyramine have shown marked reversals similar to epinephrine. Ephedrine, neosynephrin, amphetamine, privine and arterenol have also been reversals similar to epinephrine. Ephedrine, neosynephrine, amphetamine, privine and arterenol have also been reversed. Propadrine, paredrine and paredrinol have been reversed but only diminution or blocking of the pressor response is usually obtained with these compounds. The pressor response to pitressin and the depressor response to isopropyl epinephrine are unaffected by the sympatholytic agent. With increasing doses of the amines, decreasing pressor responses precede reversal.

**Actions of podophyllum derivatives.** D. G. WENZEL (by invitation), O. S. ORTH, R. T. CAPPS (by invitation), and A. H. UHL (by invitation) (*Depts. of Pharmacy and Pharmacology, University*

*of Wisconsin, Madison*). Recently the resin of *Podophyllum*, a cathartic obtained from *Podophyllum peltatum* Linné, has received attention for its effects on body cells, particularly in mitotic division, and on venereal warts. From the resin various compounds may be isolated by the use of solvents. Certain of these compounds, including a yellow Quercetin pigment and podopodophyllin, have been tested on mice, rats, rabbits and dogs for acute and chronic toxicities, and effects on the respiratory, cardiovascular and hemopoietic systems. Difficulties have been encountered in obtaining suitable solutions or suspensions with small enough particle size to permit intravenous administration of all the fractions. Toxicities based on oral, intraperitoneal or intravenous routes of administration will be presented for certain of the species. It appears that for the Quercetin compound, initial toxic effects are due to actions on the respiratory system, with marked circulatory depression soon following. Dosages repeated at intervals of ten or fifteen minutes show evidence of tachyphylaxis. Clinical actions of the individual fractions of *Podophyllum peltatum* L. will be reported.

**Studies on diffusion respiration. VI. Changes in kidney function in dogs.** R. W. WHITEHEAD, D. L. G. BLSHORE (by invitation), W. B. DRAPER, JOSEPH N. SPENCER (by invitation) and THOMAS M. PARRY (by invitation) (*From the Department of Physiology and Pharmacology, University of Colorado Medical Center*). Preliminary observations on ten dogs in diffusion respiration under pentothal (thiopental) in which urinary output was recorded, revealed that anuria developed in all animals during respiratory arrest. In an additional series of ten dogs under thiopental anesthesia, alterations in kidney function were studied. Urinary output, chloride excretion, urinary and femoral venous blood pH and arterial blood pressure were recorded before, for fifty minutes during, and following prolonged respiratory arrest. Five of the animals were not given additional fluids, the other five were given an infusion of normal saline intravenously throughout the experiment. **Results.** Anuria developed in all dogs during respiratory arrest. Often this occurred promptly with the cessation of respiration. There was no appreciable difference in the time of onset of anuria in the hydrated and non-hydrated groups. In the non-hydrated group the urinary chlorides rose from an average control value of 187 mg per cent to 517 mg per cent two hours after respiration was resumed. In the hydrated group the chlorides fell from a control of 3090 mg per cent to about 25 mg per cent. Urine flow returned within an average period of 72 seconds after respiration was resumed. Arterial blood pressure values were sufficient to provide an effective glomerular filtration pressure throughout the experiments. Femoral venous blood pH fell from

an average control value of 7.33 to 6.67 at forty-five minutes of respiratory arrest. The average pH at the end of 1½ hours post diffusion was 7.31. Urinary pH changes paralleled those of the blood during the pre and post diffusion periods.

The effect of "denervation" on the response of intestinal fistulae to several drugs. R. M. WHITLOCK (by invitation), H. L. TIECHER (by invitation), and M. H. SLEEVES, *Department of Pharmacology, University of Michigan*. Observations were made on the response of serial innervated and "denervated" Thiry-Vella jejunal fistulae in normal, unanesthetized dogs. The finding of Herrin and Miek that distention of innervated loops at 90 mm Hg pressure reflexly induces vomiting and other signs of intestinal obstruction was confirmed. The "denervated" loop is more sensitive (3-4 times) than the innervated loop to the inhibitory action of epinephrine, as noted previously by Youmans. We have found a quantitatively similar sensitivity in the "denervated" loop to the inhibitory action of atropine and tetraethyl ammonium. If it is assumed that the normal functional activity of the intestine is a resultant of the opposing excitatory and inhibitory actions of the two autonomic divisions, and that the intestine is constantly influenced by a circulating epinephrine like substance, then the observations on atropine and tetraethyl ammonium indicate that, whereas so-called "denervation" of intestinal loops by section of all nerve fibers at the vascular pedicle results in complete isolation of the structure from all central nervous connections such a procedure does not effect real denervation but only results in (1) complete loss of sympathetic innervation (after degeneration of the postganglionic fibers) (2) Section and degeneration of the preganglionic fibers of the parasympathetic division, the ganglion and postganglionic fibers remaining intact (3) Sensitization of sympathetic effector cells and probably sensitization of parasympathetic ganglia as noted by Rosenbleuth and Cannon for the metacarpal membrane.

Reactions of chronic decorticated dogs during a cycle of addiction to methadon. ABRAHAM WIKLER, *Research Department, U. S. Public Health Service Hospital, Lexington, Kentucky*. In 2 chronic decorticated dogs subcutaneous injection of 2.0 to 5.0 mg/kg of methadon ("amidone", "10820") regularly reduced spontaneous activity, depressed sham rage responses to pinching or restraint, elevated tooth pain reaction threshold, lowered body temperature and cardiac rate. In one such preparation, 43 days after completion of decortication, methadon was injected subcutaneously every 6 hours for 60 days, the dose being increased rapidly from 2.0 to 5.0 mg/kg. A high degree of tolerance developed to the analgetic, sham rage depressant and temperature low-

ering effects, but tolerance to the depressant effects on spontaneous activity was less marked. As addiction progressed pre injection restlessness and hyperirritability were observed. Two attempts at abrupt withdrawal were abandoned because the preparation exhibited extreme restlessness, marked loss of irritability, profuse salivation, vomiting, tachycardia, fever, weak pulse and gasping respirations. After a 2 day withdrawal, restlessness, hyperirritability, persistent rooting and gnawing at the floor of the circular cage were exhibited for about two weeks after which there was a gradual return to the preaddiction state. In another preparation, 6 months after completion of decortication, methadon 2.0 mg/kg was substituted for morphine after tolerance to the latter had been established. This dose was injected subcutaneously every 6 hours for 34 days. Partial cross tolerance to morphine was observed. On abrupt withdrawal of methadon, an abstinence syndrome was observed which was milder but qualitatively the same as that described above. The methadon abstinence syndrome in both preparations was indistinguishable from that of morphine.

Effects of electroshock convulsions on chronic decorticated cats. ABRAHAM WIKLER and KARI FRANK (by invitation), *Research Department, U. S. Public Health Service Hospital, Lexington, Kentucky*. In chronic decorticated cats convulsions were induced by passage of 200-450 milliamperes of 60 cycle A.C. from vertex to palate. Immediately after each seizure and for ½ to 4 hours afterward sham rage responses (chiefly facio vocal) to pressure pain stimuli applied to the tail were markedly reduced while other sham rage responses (springing, clawing, lashing of tail) evoked by non-noiceptive stimuli were unaffected or enhanced. In 3 of the preparations licking responses to tactile stimulation of the perineal region were temporarily depressed after each seizure. Righting reflexes returned within a few minutes after each convulsion while pulse rate and rectal temperatures were not altered significantly. Apnea occurred during the seizures and was followed by transient hyperpnea. No cumulative or new effects were observed after daily electroshock convulsions for 5 to 9 days. In terminal experiments the preparations were curarized and maintained on artificial respiration. Electrical activity was recorded from vertex and sphenoid screw leads immediately after electroshock induced as above. The electroencephalographic patterns were characterized by bursts of 6-21 per second rhythms interrupted by silent intervals finally terminating after a more prolonged 15-18 per second rhythm. In places fast and slow wave sequences were seen. This was more marked in preparations which were morphinized prior to electroshock. Neuropathologic studies of the remaining brains revealed no residual neocortex

The microscopic changes did not differ in degree or kind from those seen in other chronic decorticated cats which were not subjected to electroshock convulsions

**Subtilin in blood after parenteral administration** ROBERT H. WILSON, J. C. LEWIS and E. M. HUMPHRIES (by invitation) *From the Pharmacology Division and the Western Regional Research Laboratory of the Bureau of Agriculture and Industrial Chemistry, U. S. D. A., Albany 6, California* Subtilin, subcutaneously, has very low toxicity, 3 gm/kg of purified subtilin does not kill mice (Anderson and Chin, Wilson, unpublished), due to the physical characteristics of the substance. In physiological saline or serum its solubility does not exceed 0.05-0.06 gm/100 ml. Subcutaneously administered subtilin precipitates at the site of injection. Intramuscularly administered subtilin likewise is precipitated and absorbed slowly. Amounts up to 100 mg/kg have been injected into the thigh muscles of rabbits and cup plate assay of periodic blood samples has indicated no more than 2 ppm of subtilin in whole blood. Examination of the injection site 24 hours after administration revealed heavy deposits of subtilin, and a blood assay 1 month after injection showed a trace of subtilin, indicating that absorption was still in progress. Intravenous administration produces blood levels of some magnitude. A dose of 10 mg/kg, given as a 1% solution in 5% glucose in the ear vein of rabbits, gives a blood concentration of approximately 100-200 ppm in 5 minutes, dropping to 10-30 ppm in 2 hours and to zero in 24 hours. Greater dosage may kill by interfering with circulation between heart and lungs, presumably by embolism. The intravenous LD<sub>50</sub> for rabbits is not known accurately, for mice, it is about 100 mg/kg (Chin). Intravenous infusion (20 mg/kg/hr) was given a rabbit for 4 hours without producing visible symptoms, and leading to a final blood level of about 750 ppm.

**The effect of antu on the body temperature in the rat** MARTIN M. WINBURY and JOHN LOVL (introduced by W. E. Hamberger) *From Lehigh Valley Laboratories, Easton, Pennsylvania* In rats a single oral dose of ANTU ( $\alpha$ -naphthylthiourea) provokes a rapid fall in body temperature which frequently begins as early as 15 minutes after administration. Within 9 hours following a sublethal dose (0.94 to 1.88 mg per 100 gm) the temperature is reduced about 2.5°C, returning to normal 3 to 5 hours later. On administration of a lethal dose of ANTU (3.75 to 15 mg per 100 gm), the body temperature falls rapidly during the first 4 hours from the control of 37.4  $\pm$  0.14°C to about 33°C and then more gradually to the low of 29.6  $\pm$  0.51°C at death. It has been well established that the rat will develop a tolerance to toxic doses of ANTU by previous administration of this sub-

stance (Richter, C. P., J. Am. Med. Assn., 129, 927, 1915). We find that a single sublethal dose not only affords protection against the lethal effects of a toxic dose but also prevents or reduces its hypothermic action.

**Effect of ouabain and digoxin on the energy-rich phosphate store of the heart** ALBERT WOLLENBERGER (introduced by OTTO KRAYEN) *Harvard Medical School* Ouabain and digoxin, administered to the dog heart lung preparation, do not cause significant changes in the phosphocreatine and adenosine polyphosphate (APP) content of the heart, as long as their action is mainly inotropic. The toxic action of these compounds, on the other hand, is associated with a progressive loss of phosphocreatine. At the onset of ventricular fibrillation, this loss amounts to about 75 per cent. The APP concentration in the poisoned hearts is not lowered more than can probably be accounted for by cardiac edema. Both poisoned and unpoisoned hearts contain only insignificant amounts of acyl phosphate. Theoretically, the observed decrease in phosphocreatine reflects changes in the turnover of the labile phosphate groups of APP. It can be explained in three ways: (1) Synthesis of APP may be retarded. Presumably this would be the result of a depression of oxidative metabolism which toxic concentrations of cardiac glycosides are known to produce in the myocardium. (2) Breakdown of APP may be accelerated. This might take place also during the non-toxic stage, but might not be apparent because of increased phosphorylation. (3) Both factors 1 and 2 may be operating.

**The effect of splenectomy upon the susceptibility of mice to infection by trypanosoma cruzi** ORLYN WOOD (by invitation), DORIS NOSHOLD (by invitation) and LLOYD D. SEAGER *Department of Pharmacology, Woman's Medical College of Pennsylvania* Splenectomized and normal mice were inoculated intraperitoneally with approximately 50,000 organisms per kg. In one series of experiments a Mexican strain of T. cruzi was used and in a second series a more virulent Brazilian strain was employed. One group of 20 splenectomized mice were inoculated within a few days of the operation. In these the peak of infection was reached more slowly and mortality was lower than in the controls. In the rest of the experiments (120 mice) 10 to 20 days were allowed to elapse postoperatively before inoculation. In these the peak of infection was reached more rapidly and the mortality was higher than in the controls. It is suggested that the inflammatory reaction from the operative procedure made the peritoneal cavity an unsuitable environment for the organism for a few days following splenectomy.

**Effects observed in dogs following the prolonged feeding of DDT and its analogues** GEOFFREY

WOODWARD (by invitation), BERNARD DAVIDOW (by invitation), and ARTHUR A NELSON *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D C*. The *o,p'* and *p,p'* isomers of DDT, technical DDT, the dehydrochloride of DDT, DDD, and the dimethoxy analogues of DDT (DMDT) were fed to dogs for varying periods of time up to 19 months in some cases. The insecticides were administered daily except Sundry in oil solutions contained in gelatin capsules. Undissolved crystalline DDT was fed in one experiment. Chemical analyses of samples of fat taken at yearly intervals from the same dogs fed various levels of DDT showed accumulation to increase with dosage, time, and administration in solution. DDD also accumulated in fatty tissues. The average survival times in days at an 80 mg/kg/day level of intake are *p,p'*-DDT, 55, *o,p'*-DDT, 37, technical DDT, 55, DDD, 80, and DDT dehydrochloride, above 120. Dogs fed DMDT at 330 mg/kg/day still survive after 120 days. At the 80 mg/kg/day levels of *o,p'*-DDT, *p,p'* DDT and technical DDT, histopathological examination showed the major effect produced by all three substances was moderate to severe liver damage made up of central atrophy or low grade necrosis, subacute hepatitis, and fatty degeneration. The same level of DDD produced slightly less liver damage and no jaundice or hemorrhages but did cause a marked atrophy of the adrenal cortex. No significant pathologic changes were noted in the DDT dehydrochloride animals.

**Oxytocic drugs and dysmenorrhea.** R A WOODBURY, GEORGE P CHILD (by invitation), and R TORPIN (by invitation) *Division of Pharmacology, University of Tennessee, Memphis, and Departments of Pharmacology and Obstetrics and Gynecology, University of Georgia School of Medicine, Augusta*. Satisfactory relief was obtained in thirty-two of thirty-four dysmenorrhea patients by subcutaneous administrations of pitocin, in the remaining two the relief was incomplete. The beneficial effects included removal of extra uterine distress such as menstrual tension and headache as well as the abdominal distress.

Experiments were designed to investigate the influence of pitocin on the uterus in humans. Using the four balloon technique it was observed that pitocin increased the peristaltic effects of the uterine activity by bringing about better relaxation of the lower uterine segment between contractions and greater pressures during contractions. Previously it has been reported that under certain conditions pitocin caused dilation of the uterine vascular bed of dogs. Apparently the beneficial effects of pitocin can be explained partly by the rhythmic uterine contractions and partly by the reduced spasm of blood vessels. Rhythmic uterine

contractions facilitate blood flow to the uterus and expel debris.

Similar studies with posterior pituitary solution, pitressin and ergonovine demonstrate that these drugs tend to increase the frequency and amplitude of contraction in all parts of the uterus including the lower segments. Apparently these drugs do not bring about the same type of uterine activity as is elicited with pitocin. In addition pitressin produces vasoconstriction which in turn may contribute to the fact that injections of pitressin have elicited dysmenorrhea like distress in forty-four of forty-five patients.

These observations that dysmenorrhea distress may be relieved by a drug which facilitates rhythmic uterine activity should broaden the search for effective therapeutic agents for the cyclic distress.

**Rosaniline base (CI 677) as a prophylactic in *Schistosoma mansoni* infections in mice.** HAROLD N WRIGHT, ELIZABETH M CRANSTON, WAYNE A CHADBOURN (by invitation), ASHTON C CUCKLER (by invitation), DOMINIC DEGUISTI (by invitation) and RAYMOND N BIETER *Department of Pharmacology, University of Minnesota, Medical School, Minneapolis, Minnesota*. Mice were infected intraperitoneally with 100 to 125 cercariae of *Schistosoma mansoni* obtained from snails of the species *Australorbis glabratus*. Therapy of infected mice with rosaniline base, using the drug diet method of administration, was started at various time intervals before or after infection and continued for one to four weeks. Mice were autopsied between eight and twelve weeks after infection, at which time the number of worms in the portal and mesenteric veins and liver were counted and the relative degree of egg deposition in the liver noted. When therapy with rosaniline base in a concentration of 0.33 per cent in the diet was started one week before infection and continued for twenty-eight days no worms or eggs were found at autopsy in 90 per cent of the mice, whereas only 13 per cent of control mice were negative. With similar therapy begun two weeks after infection 80 per cent of mice were negative for worms and eggs. Shorter periods of therapy resulted in progressively decreasing activity. When therapy was withheld for more than two weeks after infection only a slight therapeutic action was obtained regardless of the length of therapy. From the results obtained rosaniline base appears to possess a high degree of prophylactic activity and a low degree of curative activity in *Schistosoma mansoni* infections in mice. The possible activity of other triphenyl methane dyes is under investigation. This work was done under contract with the Office of the Surgeon General of the United States Army, the United States Public Health Service and the University of Minnesota.

**Comparison of biological and chemical evalua-**

tions of sex hormone balance M X ZARROW (by invitation) and W T SALTER *From the Laboratories of Pharmacology and Toxicology, Yale University of Medicine, New Haven, Connecticut* In studying the urinary excretion of sex steroids chemical methods are available which include degradation products in addition to the original hormone These degradation products possess varying degrees of residual biological activity which is not indicated in the chemical analysis It is a moot point whether these break down products should be rated at their biological equivalents or in accord with the original chemical origin Accordingly studies have been made on the urine of normal human subjects and patients suffering from cancer (mammary and prostatic) and various endocrinopathies such as gynecomastia, hypogonadism, adrenal virilism and eunuchoidism Biological determinations were carried out on urine samples hydrolyzed with HCl and extracted with ether The "estroids" were assayed in castrated mice using estrone as a standard and the androgens were assayed in day-old cockerels using androsterone as a standard Chemical determinations were made by the technique of Cahen and Salter (*J Biol Chem* 152 189, 1941) for 17-ketosteroids and of Oesterling and Salter (*Fed Proc* 6 171, 1947) for "estroids" The results from the chemical determinations tend to give higher values than obtained by biological assay For example, in pooled urine from a group of soldiers with nutritional gynecomastia the chemical value for "estroids" was 8 micrograms per 24 hours as compared with 4 micrograms obtained by bioassay However, in two cases with negative values for 24 hour determination in urinary estrogens by the chemical method negative results were also obtained in the

bioassay More detailed data in various pathological conditions are reported

The effect of tetraethyl ammonium chloride on gastric secretion and motility MITCHELL ZWEIG (by invitation), F STEIGMANN, and KARL A MEYER (by invitation) *Hektoen Institute for Medical Research of Cook County Hospital and the Department of Surgery, Northwestern University, School of Medicine* Tetraethyl Ammonium Chloride (Etamon Parke, Davis Co), which has the ability to block at the autonomic ganglia the transmission of both sympathetic and parasympathetic nerve impulses, was employed in a preoperative study of 11 patients with hypertension for determination of results which might be expected following sympathectomy Because of paucity of reports on the effect of this drug on gastric functions, it was considered pertinent to study its effect on gastric motility and secretion The following procedure was used A balloon was passed into the fundus of the stomach, inflated with 10 cc of air, and then attached to a kymograph Gastric juice was aspirated through a Levine tube The total quantity aspirated in fifteen minutes was measured and titrated for both free and total acid A base level of secretion and motility was taken for at least one hour After obtaining the base level, 400 to 600 mgm of Tetraethyl Ammonium Chloride were given intravenously Within five minutes after the injection of the drug every patient had a complete cessation of motility This lasted from 15 to 100 minutes The amount of free acid in milliequivalents was determined for an equal period before and after the Tetraethyl Ammonium Chloride was given In every instance, except in four patients who secreted no free acid during the test period, the acid levels were reduced after the drug was given

## THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

### THIRTY-THIRD ANNUAL MEETING

*Atlantic City, New Jersey, March 15, 16, 17, 18, 19, 1948*

(For possible corrections in any of the following abstracts see the next issue)

The transparent chamber technique in the mouse in the study of tumor histo-physiology (Motion Picture) GLENN H ALGIRE and FRANCES Y LEGALLAIS (by invitation) *National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda, Md* The transparent chamber technique permits in vivo microscopic observation of growth and vascular development of transplanted tumors over intervals up to 100 days Examination and photography of

blood vessels and cells are carried out at magnifications up to 1000X, using oil immersion objectives The method is being applied to problems of tumor induction and growth, effect of chemical and physical agents on tumors, cell migration and division, immunity, and circulatory physiology in normal and neoplastic tissues The motion picture illustrates the procedures for operation and examination of the living tissues

Discrepant animal and clinical observations on

**hypothermia and procaine** FREDERICK M. ALLEN  
*City Hospital, Welfare Island, New York, N. Y.*

Different temperature reactions of different species result in discrepancies regarding treatment of conditions such as burns and visceral shock, but reduced temperature is supported by the general evidence. Laboratory animals react to procaine differently than man to such an extent that there is doubt of adequate duplication of the recent clinical uses of this drug, on a physiological basis. The new concepts of certain pathological processes may be still more difficult to elucidate in the laboratory.

**The relationship of caloric intake level and protein intake level to rate of protein synthesis** EARL P. BENDITT, *Department of Pathology, The University of Chicago Clinics, Chicago*. The purpose of these experiments was to determine the influence of caloric and protein intake levels on the rate of tissue synthesis in protein depleted animals. Standard protein depleted animals were used. The method of protein depletion and repletion have been described (Wissler, Woolridge, Steffee and Cannon, *J. Immunol.* 52:267, 1946). Caloric intake was varied at constant protein intake, protein intake was varied at constant caloric intake and the two were varied together. The protein was lactalbumin and casein (half and half) and the rations were adequate in minerals, vitamins, roughage and fats. Five or six animals in individual cages were fed each ration for a period of 14 days. They were then sacrificed and the carcasses analyzed for protein, fat, water and ash. The intakes were calculated as Cal./m<sup>2</sup>/day and gm. of protein/kgm. body weight/day, and rates of protein fabrication as gm. protein gained/kgm. body weight/day. The results were as follows: 1) Limitation of caloric intake below 1240 Cal./m<sup>2</sup>/day restricted protein utilization. 2) Caloric intakes above this level did not augment protein synthesis, but were associated with increased deposition of fat. 3) With an adequate caloric intake the rate of protein utilization increased with rising levels of protein intake over the range studied (up to 14.6 gm./kgm./day). 4) Growing and mature protein depleted animals were found to react in an almost identical fashion.

**Production of intimal atherosclerosis by intravenous injection of colloidal cholesterol into rabbits** MARGARET BEVANS, LIESE LEWIS ABELL, and FORREST E. KENDALL (introduced by H. P. SMITH). *From the Research Service, First (Columbia) Division, Goldwater Memorial Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University*. Colloidal solutions containing 2.5% cholesterol stabilized with 1% sodium stearate can be rapidly injected into the ear vein of rabbits without producing adverse symptoms. The injection of 0.5 gm. of cholesterol into a 2.5 kg. rabbit leads to an increase in the serum cholesterol level amounting to 250-300 mg. % above the initial

concentration 10 minutes after injection. The level falls to a value 100-150 mg. above the base line in 6 hours and then rises again to 150-200 mg. at the end of 24 hours. Normal levels are regained in from 4 to 7 days. Daily injections of cholesterol result in serum levels comparable to those obtained when the same amount of cholesterol is fed and lead to arterial lesions indistinguishable from those obtained in feeding experiments. Gross intimal lesions were first noted after the injection of 6.5 gms. of cholesterol over a period of 13 days. These were few and small and occurred only in the ascending aorta. Extensive lesions were produced by the injection of 14.5 gms. of cholesterol over a period of 36 days.

**Cholesterol in intestinal and hepatic lymph of the rat** JESSE L. BOLLMAN and EUNICE V. FLOCK. *Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota*. Intestinal lymph was drained continuously through a small polyvinyl plastic tube from the main intestinal lymphatic near the cysterna chyli. After a control period the rats were fed by stomach tube on the second and second and third day. Samples of lymph were collected to cover periods of 3, 6, 9, 12 and 24 hours after each meal. Following a fat free meal to which cholesterol was added the concentration of cholesterol of the lymph was increased over the control values for about 12 hours and usually returned to the control values before 24 hours. Both free and ester cholesterol increased but the greater increase was that of cholesterol esters, although considerable variation in the relative proportion of each was found. Addition of oleic acid or of neutral fat to the same diet containing cholesterol did not greatly increase the concentration of cholesterol appearing in the lymph. The amount of cholesterol absorbed via the lymph is extremely small compared to the lymphatic absorption of fat. The concentration of cholesterol and of cholesterol esters in hepatic lymph is similar to that of plasma and the fluctuations are much smaller than in intestinal lymph.

**Blood iodine in alloxan diabetes of dogs** H. G. DAVIS, JR. (by invitation) and R. D. BAKER. *Department of Pathology, The Medical College of Alabama*. Total blood iodine studies were done on two groups of eight dogs using Steven's acid ashing method. Six of each group were made temporarily or permanently diabetic with alloxan. One group received food containing 0.5 mgm. of iodine daily. The other did not. Two dogs of each group were retained as controls. In every case a rise in blood iodine was obtained where there was sufficient alloxan dosage to cause the blood sugar to rise. The blood iodine remained high in the dogs which became permanently diabetic. The values for those animals receiving iodine supplement rose considerably higher than those which did not. Theories suggested to explain the rise in blood iodine include: (1) Damage to the kidneys by alloxan with

failure of excretion (2) Damage to the liver with failure of hepatic excretion of iodine into the intestine (3) Removal of endocrine action of islet tissue on the thyroid, allowing excessive excretion of iodine by the thyroid

**Iron absorption in normal subjects and in patients with anemias of varied etiology** REUBENIA DUBACH (by invitation), SHILILA CALLINDER (by invitation) and CARL V. MOORE *Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.* Measurement of iron absorption by the isotope technique has proved difficult because not all of the iron absorbed from an oral dose may be utilized for hemoglobin formation. It has become necessary, therefore, to alter the procedure so that retention could be divorced from utilization. Accordingly, the method has been extended to include determination of the unabsorbed radioiron in feces as well as that converted into hemoglobin. Recovery of radioiron from feces was accurate within 10%. The test dose equalled 1 mg./kg. body weight and was given as ferrous chloride. In iron-deficient subjects, all of the radioiron not recovered in the feces was utilized promptly to form hemoglobin. Results in normal persons indicated that a small amount of absorbed iron was not converted into hemoglobin, the amount recovered in feces plus that found in blood equalled 83 to 100% of the test dose. In 12 subjects with fever, untreated pernicious anemia, refractory anemia, and hemolytic anemia there was a wider discrepancy between the amount of iron retained and the amount utilized, and some of these patients absorbed a relatively large amount of the metal. For instance, 2 patients with Hodgkin's disease and an associated hypochromic anemia retained 52 and 32%, respectively, yet utilized only 15 and 2.5%. In 3 patients with untreated pernicious anemia, the proportion of retained iron which appeared in the blood was small at first, but increased sharply after liver therapy. Fecal recoveries indicated that they had absorbed from 15 to 33% of the test dose. From these results, it is concluded that 1) the per cent of a test dose of radioiron which appears as circulating hemoglobin should not be accepted as a measure of the total amount absorbed except in afebrile patients with hypochromic anemia, and 2) if iron absorption is regulated by the degree of "block" present in mucosal cells of the intestinal tract, as seems to be the case, the block is only relative and is not nearly as efficient or complete as earlier work had indicated.

**A study of experimental serum sickness** WILLIAM E. EHRLICH, CAROLYN FORMAN (by invitation) and JOSEPH SEIFTER *Philadelphia General Hospital, University of Pennsylvania, Wyeth Institute Applied Biochemistry*. Serum sickness was produced in rabbits by injecting them with horse or duck serum. It was confirmed that large doses of

salicylates inhibit the production of certain phenomena of this condition. As these doses caused both an alarm reaction and an anticoagulant effect, the role of these phenomena in serum sickness was investigated by treating serum injected rabbits with alarming stimuli such as colchicine, or with anticoagulants such as dicoumarol or algarin (a heparin like compound). The results of these studies will be presented.

**Respiration of tissues from hypersensitive animals when antigen is added in vitro** RICHARD H. FOELLIS, JR. *Department of Pathology, Johns Hopkins University*. The anaphylactic type of hypersensitivity (Arthus phenomenon, protein type hypersensitivity) is characterized by contraction of smooth muscle and damage to endothelium of blood vessels. Whether the local Arthus reaction which can be elicited in heart, kidney, testis, etc. is due entirely to vascular damage is not clear. Biochemical studies of isolated tissues would seem to offer an approach to this problem. Guinea pigs and rabbits were injected respectively with egg albumin and horse serum. At appropriate intervals the animals were tested intracutaneously with antigen. When they were found to exhibit a strong Arthus phenomenon, the oxygen consumption of various tissues—liver, kidney, smooth muscle, heart muscle and testis, was then determined manometrically using Krebs-Ringer solution with or without added homologous serum as substrate. Specific antigen or control material was added from the side arm after equilibration and hourly readings made up to six hours. Experimental data indicate that there is no appreciable change in the oxygen consumption (in micro liters) per mg. tissue (dry weight) per hour from normal when specific antigen is brought in contact with cells of the organs tested. Such findings would confirm and extend observations heretofore recorded.

**Histochemical studies of basement membranes** ISIDORE GERSH *Department of Pathology, University of Illinois College of Medicine, Chicago*. Basement membranes in skin, developing kidney, thyroid gland, and lung of normal organs have been studied after staining for reticular fibers and for an optically homogeneous component. The latter has been found to be a carbohydrate-containing protein, which can be dissolved or destroyed by certain reagents. These also affect the "ground substance" of the connective tissue in a similar way. The "ground substance" and the homogeneous component of the basement membrane are morphologically continuous. During histogenesis, as well as in inflammation, morphological changes take place in the homogeneous component which suggest that the basement membrane may be a highly labile, modifiable structure.

**Sodium-potassium levels and adrenal gland necrosis in experimental simian malaria** ALFRED



GOLDEN and R. R. OVIKMAN (by invitation) *Departments of Pathology and Physiology, University of Tennessee, College of Medicine, Memphis, Tennessee* Monkeys experimentally infected with P knowlesi show an average survival when untreated of five days. During their illness they develop profound alterations of blood potassium and sodium levels as determined by flame photometry. Associated with these and other biochemical changes there is consistent focal necrosis of the adrenal gland cortex which varies from focal lesions in all zones of the cortex to large patches of such necrosis associated with hemorrhage. An entirely similar lesion was demonstrated at autopsy in a human case of P vivax infection.

Adrenal gland lesions in experimental simian malaria and similar human lesions in varied diseases. ALFRED GOLDEN, *Department of Pathology, University of Tennessee, College of Medicine, Memphis, Tennessee* Lesions entirely analogous to those seen in the adrenal glands of monkeys dying with acute P knowlesi infection were observed in the following diseases at post mortem examination: B pyocyaneus endocarditis, healing impetigo and fatal diarrhea in a newborn infant, disseminated tuberculosis in an adult, acute meningococcal meningitis and septicemia, multiple gunshot wounds with multiple perforations of the gastrointestinal tract, acute gangrenous cystitis and acute gangrenous pyelonephritis, multiple abscesses of the kidney and lung, fulminant hypertension with terminal bronchopneumonia, severe body burns, acute P vivax malaria in an adult, gangrenous vaginitis following radium implantation for a carcinoma of the cervix, acute interstitial nephritis. The evolution of the lesion can be traced from its minimal manifestations to its severest forms. The lesion is identical to that seen previously by the author in epidemic typhus fever.

Susceptibility of macacus cynomolgus to Japanese encephalitis virus with special reference to the alimentary tract. F. B. GORDON, F. M. SCHABEL, JR. (by invitation) and MARGARET ABENDROTH (by invitation) *Department of Bacteriology and Parasitology, University of Chicago* As part of a series of experiments on the susceptibility of various animals to infection by feeding with neurotropic viruses, 2 cynomolgus monkeys were fed bread soaked in an emulsion of Japanese encephalitis virus. No clinical abnormalities resulted but one of the monkeys had a viremia from the 3rd to the 10th day. No virus could be recovered from oral or rectal swabs by subinoculation of mice with penicillin treated suspensions. The monkey with viremia resisted a later intracerebral inoculation of virus (18,000 mouse infectious doses) which produced severe encephalitis in the other fed monkey and two controls. In the 3 latter monkeys, infected by intracerebral injection, viremia

occurred early but terminated on the fourth to the 7th day, but the time of the onset of illness. Oral and rectal swabs were tested for virus at intervals between the time of inoculation and death. No virus was found in the rectal swabs, but it was demonstrated once in small amount in the mouth on the 5th day after intracerebral inoculation. The 3 monkeys were killed when severely affected, and some 18 tissues of each were tested for virus. It was recovered from the central and peripheral nervous systems, from leg muscle, and from the anterior end of the alimentary canal, but none was found in the intestine.

Failure of trypanosoma cruzi lysate in treatment of Brown-Pierce carcinoma of rabbit. O. M. GRUNZIR and R. A. FISHLIN (by invitation) *Research Laboratories, Parke, Davis, & Co., Detroit, Mich.* A non virulent Trypanosoma cruzi strain was grown on beef blood in heart brain broth effusion agar medium for fourteen to sixteen days. The trypanosomes were harvested by centrifugalization, yielding a sediment of five billion organisms per cubic centimeter. A distilled water lysate, 1:50 with 1:5,000 dilution of cetyl trimethyl ammonium bromide, each cubic centimeter of which contained about 100 million organisms, was prepared from a 72 hour T. cruzi harvest, kept at -6°C. A group of nine rabbits was inoculated intratesticularly with Brown-Pierce rabbit carcinoma cell suspension. Three days after inoculation, five animals received intramuscularly 1.0 cubic centimeter each twice daily for seventeen days of the T. cruzi lysate. In eight days, the treated and untreated rabbits showed nodular growth in the testes. On the tenth day, the consolidated testis of one treated rabbit was excised and part of the emulsified material was injected into the testis of each of four normal rabbits, which eight days later showed neoplastic growth. At the end of seventeen days of treatment with the lysate, the treated and untreated rabbits presented extensive nodular consolidation of the testes. On the twenty-seventh day, three treated rabbits showed neoplastic metastasis in one or both eyes, including the one from which the tumor-bearing testis was removed, but none occurred in the control rabbits. The treated and two control rabbits showed massive tumor growths in the testes. In the other two control rabbits, the tumors had regressed. Under our conditions, the use of T. cruzi lysate had no inhibitory effect on the growth of the Brown-Pierce carcinoma in rabbits.

Tumor therapy by the direct infiltration of radioactive colloidal metallic gold. P. F. HAHN *Vanderbilt University School of Med., Nashville, Tenn.* If the list of available radioactive isotopes is carefully considered from a standpoint of potentially useful therapeutic agents, keeping certain logical criteria in mind, it is found that there are probably less than ten which would be suitable. These

criteria include 1 Desirable radiation spectrum, 2 Known biological behaviour, 3 Suitable half-life, 4 Known chemical behaviour, 5 Good cross section for bombarding particles used, 6 Absence of long-lived contaminants, and 7 Economical production Gold<sup>198</sup> made in the chain reacting uranium pile by absorption of slow neutrons by the 100% abundant isotope Au<sup>197</sup> appears to meet these criteria to a degree not approached by other isotopes. In addition it has specific properties such as its metallic colloid's insolubility in all body tissue fluids which render it particularly valuable as a therapeutic agent. By means of a needle of fine calibre this material may be infiltrated directly and throughout a tumor mass in the same manner one would use novocaine. It has been found that when administered in this manner the colloid remains at the site of infiltration. Where is in the use of radium needles and radon seeds it is necessary to filter out the alpha and beta radiation in order to prevent sloughing at the site of application due to over intense localized radiation effects, and to rely only on the gamma radiation, using the gold isotope one makes use of the beta radiation from not six or eight sources but from many billions of point sources. Thus the ionizing radiation is delivered uniformly throughout the desired mass of tissue and adjacent structures are unaffected since the mean path of the beta particles is a millimeter or less. Results of clinical application will be presented.

**The renal lesion of murine haemobartonellosis compared with lower nephron nephrosis** ROBERT S. HAUKOHL (introduced by W. A. D. ANDERSON) *Department of Pathology and Bacteriology, Marquette University School of Medicine, Milwaukee, Wis.* The renal changes were studied in 34 rats dying in the acute stage of haemobartonellosis following splenectomy and the inoculation of infected blood. The renal lesion is characterized by 1) marked degeneration, necrosis and desquamation of the epithelium of the distal convoluted tubules, 2) accumulations of hyaline droplets in the epithelium of the distal convoluted tubules, 3) precipitation of heme pigment casts and crystals in the tubules of the lower nephron and collecting tubules, and 4) minimal structural change of the glomeruli, upper and intermediate nephrons and interstitial tissue. The heme pigment casts and crystals and the hyaline droplets stain positively with the hemoglobin stain of Dunn and Thompson (*Arch. Path.* 39:49, 1945), and fail to stain with Prussian blue. Two factors are essential for the production of this lesion: 1) relative renal anoxia secondary to shock, anemia and the vaso-constrictor action of circulating free hemoglobin, and 2) the excretion of large amounts of hemoglobin with precipitation in the lumen (casts and crystals) and reabsorption by the tubular epithelium (hyaline droplets).

The renal lesion of haemobartonellosis is essentially similar to the renal changes in human lower nephron nephrosis. The appearance of hemoglobin positive hyaline droplets in rat kidneys and not in the human counterpart may be attributed to the relatively large amount of hemoglobin excreted in haemobartonellosis. The absence of interstitial reaction is due to the relatively short interval between the onset of hemoglobinuria and death.

**Protection against bacterial endotoxin by penicillin and its impurities** WALTER D. HAWK (by invitation) and C. PHILLIP MILLER *Department of Medicine, The University of Chicago, Chicago, Illinois* The protective action of pure penicillin against a number of bacterial endotoxins, already reported, has been increased by the addition of certain substances present in impure preparations of penicillin. Mice were treated with three intraperitoneal injections of 0.5 ml. of the penicillin preparation during the 24 hours preceding intraperitoneal injection of bacterial endotoxin (S. Vertrycke). About 10,000 units of penicillin per mouse were used. Untreated animals received physiological saline and other control solutions before their injections of endotoxin. Treatment with crystalline penicillin increased the LD<sub>50</sub> of endotoxin to about twice that obtained in controls. Treatment with the impure penicillin increased the LD<sub>50</sub> to more than six times that obtained in the controls. Treatment with the impurity, unaccompanied by penicillin, provided no protection against the endotoxin. The effective impurity is water soluble, filtrable, heat stable, resistant to acidification, and soluble in certain organic solvents.

**Influence of various protective compounds in preventing fatty changes by dichloroethane** BENJAMIN HIGHMAN *National Institute of Health, Pathology Laboratory* Several series of white male rats of the Sprague Dawley strain, weighing approximately 90 grams, were injected subcutaneously with 1 ml./kg. of undiluted dichloroethane. The mortality rate was low, but the rats developed severe though transitory fatty degeneration of the liver, kidney and heart, as shown by frozen sections stained for fat. These changes were usually marked in rats killed 24 hours after the injection. When 1000 mg./kg. of dl-methionine in 2 per cent aqueous solution was injected intraperitoneally immediately after the dichloroethane, these changes were much less marked and were minimal or absent when an additional 500 mg./kg. of methionine was injected 5 hours later. The results were reproducible. Larger rats were relatively more susceptible to dichloroethane and diluting the compound with oil increased its toxicity often leading to considerable mortality even after methionine administration. Various agents

were tested, using as controls groups of unprotected and methionine protected rats simultaneously injected with dichloroethane. No compounds tested equaled methionine in potency. Slight protection was offered by some compounds containing an amino group in a benzene ring like sulfanilamide (given by stomach tube) and aniline. Usually the heart was most readily protected and the liver least. The results are consistent with previous findings based on the ability of such compounds to reduce mortality. It is suggested that such histologic methods be used to supplement other methods for testing the efficacy and mode of action of various substances for protection against other toxic agents producing transient histologic changes.

Arterial disease may be a matter of days, not decades. **RUSSELL L. HOFFMAN**, Department of Pathology, L S U School of Medicine, New Orleans. There is a growing body of evidence incriminating certain lipid substances under certain conditions as an important factor in the pathogenesis of arterial disease. This applies regardless of species, but the form the arterial lesions take varies widely in different species and is apparently conditioned by the pattern of lipid metabolism characteristic of the species. For this reason it seems propitious at this time to shift emphasis from anatomical form to some of the broader aspects of pathological physiology that may be involved in the genesis of arterial disease. Data previously presented to this society have shown that arterial lesions can be produced with regularity in dogs by feeding a specified high fat diet for two months or longer, than damaging their kidneys in any of several ways. These lesions, which constitute another example of arterial disease related to a disturbance in lipid metabolism, have been observed as early as four days after the production of renal insufficiency. These studies have suggested that arterial disease may be a matter of days, not decades. Several human cases consistent with this suggestion are presented, and some of the implications and possible applications of the suggestion are discussed.

**Failure of implantation in vitamin E deficient rats.** **HANS KAUFITZ** and **CHARLES A. SLANETZ** (by invitation). From the Departments of Pathology and Animal Care, College of Physicians and Surgeons, Columbia University, New York City. A colony of highly inbred rats was kept for seven generations on a purified vitamin E deficient diet containing 10 per cent lard. Adult animals therefore consumed roughly 30 mcg tocopherol daily because lard was the only measureable source of tocopherol. The controls obtained the same ration supplemented by 3 mcg synthetic dl alpha tocopherol, (Hoffmann La Roche) per 100 gms diet. 721 mating experiments, carried out

between the ages of 5 weeks and 20 months, revealed that after the 8th week the rate of implantation in the deficient group was significantly lower (chi square) than in the controls. The differences persisted when the body weight of the animals was taken into account. It seems that there exists a specific tocopherol requirement for implantation steeply increasing with age. A daily tocopherol intake of roughly 30 mcg (as provided by the basic ration) enabled 50 per cent of the 2-4 months old animals to become pregnant. The requirements for implantation represent only a fraction of those necessary for normal gestation and lactation. Experiments are being performed which may clarify whether the implantation failure in vitamin E deficiency is caused by ovarian or uterine dysfunction or by disturbances of the fertilization or the passage of the ovum from the ovaries to the uterus.

**Effect of intravenous injection of oxidized cholesterol upon the production of atherosclerosis in rabbits.** **FORREST E. KENDALL**, **WALTER MEYER**, and **MARGARET BEVANS** (introduced by H. P. SMITH). From the Research Service, First (Columbia) Division, Goldwater Memorial Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University. Bergstrom and Wintersteiner (J Biol Chem 141, 597, 1941) showed that colloidal solutions of cholesterol are readily oxidized by air to give 7-keto cholesterol and 7-hydroxy cholesterol as the principal products. Intravenous injection of these oxidized solutions into rabbits leads to blood cholesterol levels comparable to those obtained with the unoxidized preparations. However striking differences are found in the immediate effect of the two preparations upon the arterial walls. Lipid droplets can be detected within the cells of the intima 24 hours after a single injection of 0.5 gms of oxidized cholesterol. 72 hours later proliferation of the intimal cells can be seen. These lesions persist without much change for at least 6 months. Multiple injections lead to an immediate increase in the amount of sudanophilic material in the intima which roughly parallels the amount of oxidized cholesterol injected. These early changes are not produced by the injection of unoxidized cholesterol. However if high serum cholesterol levels are maintained for several weeks by the injection of either material there is little difference in the extent or character of the lesions produced.

**The production of phage in the absence of cellular growth.** **A. P. KRUEGER**, **T. COHN** (by invitation) and **P. N. SMITH** (by invitation). From the Department of Bacteriology and Office of Naval Research Task V, University of California, Berkeley, California. Reproduction of the cellular sub-

strate is generally considered to be an essential condition for the formation of phage although some evidence has been secured indicating that the two process may be separated. We have described elsewhere the accelerating action of penicillin on phage engendered lysis of Staphylococci and in the present experiments, we have utilized this effect to impose conditions such that [phage] increases without detectable bacterial growth. Actively growing Staphylococci are centrifuged from the medium and are resuspended in Locke's solution, at 5°C, containing 10 unit of penicillin G/ml after 10 hour, phage is added to the suspension using a volume which does not introduce a significant amount of nutrients. The mixture is kept an additional 10 hour at 5°C and is then shaken in a water bath at 36°C [bacteria] is determined at brief intervals throughout the reaction by means of direct microscopic counts and nephelometric measurements in the Klett-Summerson photoelectric comparator. After lysis is complete (about 15 hours after removing the suspension to the 36° bath) [phage] is determined by Gratia's plaque count method. Starting with  $1 \times 10^8$  plaques/ml, the titre after lysis is found to be between  $4 \times 10^8$  and  $5 \times 10^8$  plaques/ml [bacteria] remains constant until massive lysis begins about 0.6 hours after bringing the suspension to 36°C.

**The effect of bacterial endotoxins on the carbohydrate metabolism of the rabbit** ERNEST KLIN (introduced by C. PHILLIP MILLER) *Departments of Pharmacology and Medicine, University of Chicago, Chicago*. Rabbits were injected intravenously with a dose of meningococcal or Salmonella endotoxin, which caused death in 2-3 hours. The blood glucose showed a rapid increase, followed by hypoglycemia. Blood inorganic phosphate and lactic acid increased, while blood pyruvic acid diminished. There was a marked decrease in liver and muscle glycogen. Liver and muscle lactic acid increased, while pyruvic acid diminished. Succinic dehydrogenase was significantly inhibited in both liver and muscle. Cytochrome oxidase was not affected. (Supported by a grant from the U. S. Navy, Office of Naval Research.)

**Plasma tocopherol levels in cardiac patients** JANET M. LEMLEY (by invitation), ROBERT G. GALE (by invitation), ROBERT H. FURMAN (by invitation), MARY E. CHARRINGTON (by invitation) and GEORGE R. MENEELY. *From the Departments of Medicine and Biochemistry of Vanderbilt University School of Medicine*. The importance of tocopherol in maintaining normal muscular function has been established. Cardiac manifestations have been described in vitamin E deficient animals. Claims have been made of benefit to cardiac patients

following tocopherol administration despite lack of evidence that vitamin E deficiency exists in humans. Twenty five cardiac patients were studied for evidences of tocopherol deficiency. The tocopherol content of the blood plasma was determined by the method of Quarte and Harris. Normal values by this method average 1.15 mg per 100 ml of plasma. The lower limit of normal is not firmly established. Some consider 0.88 mg % as this limit, while others place the lower limit of normal at 0.61. The mean plasma tocopherol level for the group studied was lower than the normal mean. Several patients exhibited sufficiently low plasma tocopherol levels to suggest deficiency. The evidence presented does not indicate a general deficiency of tocopherol in cardiac patients but suggests that in some patients with heart disease tocopherol deficiency might be a factor.

**On the demonstration of hyaluronidase in cercariae of schistosoma mansoni** MILTON D. LIVING, RAY F. GARZOLI, ROBERT E. KUNTZ and JOHN H. KILLOUGH, (introduced by K. M. ENDICOTT). *Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland*. Hyaluronidase in relatively high concentration has been shown to be present in solutions derived from suspensions of cercariae of *S. mansoni* incubated at 25°C for 18 hours. To demonstrate this activity sodium hyaluronate must be included with the suspensions. Enzyme activity was uniformly present although found to vary considerably. The relationship between penetration of cercariae through the ground substance and enzyme activity will be discussed. A new viscometer designed specifically for viscosity ranges suitable for hyaluronidase assay was used for determinations in this study. Sodium salicylate given *per os* to mice over a seven day period failed to appreciably alter the number of adult worms recovered after exposure to known numbers of cercariae. Studies on parenteral administration of this salt in high concentration are now in progress.

**Experimental production of acute pancreatitis** ROLF LIVING (by invitation) and STEPHEN MADDOCK. *From The Surgical Research Laboratory of the Boston City Hospital*. At the last meeting of this society a preliminary report on the production of acute pancreatitis was presented. Since that time the studies have been completed. A series of 100 cats, including controls, were subjected to ligation of both pancreatic ducts and the pancreas stimulated in a variety of ways: pilocarpine, eserine, acetylcholine and secretin with and without food. In the majority of above animals gross fat necrosis was found at the end of 24 to 48 hours. The amount of fat necrosis varied greatly, being most marked in animals which had been fed high fat meals prior to duct ligation. In control animals in which the

ducts were exposed by laparotomy no changes were observed at 18 hours whether the animals were starved, fed or chemically stimulated. The microscopic findings showed considerable variation in inflammatory reaction from edema with exudate and inflammatory cells to areas in which an entire lobule of the gland showed dissolution. Frank vascular lesions were not encountered but there were patchy areas of hemorrhage mingled with inflammatory reaction in a number of instances. Some sections showed damage to the walls of the blood vessels but in no instance was a gangrenous pancreatic necrosis observed. It is our opinion that this condition did not occur in cats because the pancreas is thin and lying free in the mesentery of the duodenum. The human pancreas in contrast with its thickness, dense capsule and peritoneal covering offers resistance to the extrusion of dammed up pancreatic juice.

**The histogenesis of cells in pneumonia as seen in multiple lobe pneumococcus (type I) infections in dogs.** CLAYTON G. LOOSLI, *Dept. of Medicine, University of Chicago, Chicago, Illinois.* A study was made of the origin of cells in the pneumonic exudate in the lungs of a single animal subjected to a pneumococcus (type I) infection in a different lobe at daily intervals until 5 lesions of increasing ages (4, 24, 48, 72, 96 hours) were produced. Twenty-two dogs weighing 8 to 12 kilograms were employed. The inocula (0.001 to 0.03 cc. of an 18-hour broth culture of type I pneumococcus in one cc. starch) were introduced into the lungs through a radiopaque catheter inserted into the bronchi via the tracheae with the aid of a fluoroscope. The extent and site of the infections produced by each inoculation was determined by x-rays. The animals were sacrificed with intravenous injections of pentobarbital sodium. The lungs were fixed intratracheally with Zenker-formol solution after the vessels were clamped to hold the blood in the capillaries, sectioned and stained by the hematoxylin-eosin-azure II method. By this technique, the pneumonic process was shown to be a dynamic one and the kinds of cells in the exudate varied with the age of the lesions. Polymorphonuclear leukocytes predominated in the 4 and 24-hour stages. During this time, however, large numbers of hematogenous lymphocytes and monocytes also entered the alveolar exudate. These cells hypertrophied and transformed themselves into macrophages which replaced the polymorphonuclear leukocytes. The local septal cells did not desquamate to form macrophages in the early stages but gradually enlarged and became most conspicuous on the alveolar walls in the 96-hour lesion when resolution began.

**Hypoprothrombinemia with hemorrhage as a cause of death in the rat in hypervitaminosis A.** CHARLOTTE L. MADDOCK (by invitation), S. B. WOLACH and DOROTHY JENSEN (by invitation)

*Department of Pathology, Harvard Medical School and Surgical Research Laboratory, Boston City Hospital.* During the course of an investigation on the bony changes resulting from excessive vitamin A administered to rats maintained on a rachitogenic diet for 3 weeks and over, an hemorrhagic diathesis developed in 90% of cases. Previous work in this laboratory on hypervitaminosis A, done on large series of animals maintained on normal diets, had never demonstrated striking hemorrhages. In this respect, the pathology resulting from excessive vitamin A was contrary to the reports of some other workers. A synthetic diet, devised by Bessey, was utilized. It was presumably complete in all respects except for absence of vitamin D, deficient P, and excess Ca. Vitamin K was not added because it is normally synthesized in rats' intestines. Growth on this diet was equal to that of stock animals for a period of 7 weeks. Subsequently weight gains declined somewhat. Rats, 41 to 71 days old, maintained on the diet since weaning, were given excessive doses of vitamin A for 5 days or longer. Multiple massive hemorrhages developed, most commonly located in the subdural space, epididymis, skeletal muscles and body cavities. Lymph nodes were hemorrhagic in appearance because of blood-filled sinuses. Hematocrit values fell as low as 13%. Prothrombin times were prolonged (70-183 seconds, controls 25 seconds or less). It seems that the Bessey diet plus hypervitaminosis A produces hemorrhages sufficient to cause death. If blood and tissue concentration of vitamin A can be brought to sufficiently high levels, fatal massive hemorrhages definitely related to hypoprothrombinemia will result.

**Histochemical features of the renal basement membrane.** J. F. A. McMANUS (introduced by R. D. BAKER), *Department of Pathology, The Medical College of Alabama, Birmingham, Alabama.* The basement membrane (BM) of the kidney is colored with Schiff's reagent after periodic acid (PAS), a reaction originally described for mucin (Nature, Aug., 1946) which now appears to be characteristic of any carbohydrate. BM contains protein as demonstrable by digestion experiments. Fat shown with sudan IV or sudan black is absent normally but appears sometimes in disease. The BM is preserved in sections after almost any type of handling. The BM does not autolyze for a long time, suggesting the absence of any inherent enzyme synthesis. Accordingly, no phosphatase activity is shown in the BM of the human kidney at pH 4 to pH 9.5 with glycero-phosphate, nucleic acid, adenylic acid, hexose diphosphate or lecithin as substrates except where the overlying epithelium is positive. The BM does not digest with diastase or hyaluronidase, not containing glycogen or hyaluronic acid in available form. In one case in which it was possible to study

the mitochondria of the tubule cells and the tubular BM, colloid droplets, replacing the mitochondria, were covered by thickened and abnormal BM. When the glomeruli are obsolescent or obsolete the corresponding tubules become atrophied and collapsed, with pigmentation of the epithelial cells in many instances. The BM becomes wrinkled and thickened, frequently duplicated and shows striking abnormality with PAS. These observations suggest a passive role for the BM, closely related to the activity of the tubular epithelium. It is likely that the BM is of the nature of chitin, as Lillie suggests, this is being tested with glycosidase.

**The fate of aged leukocytosis-promoting factor of exudates** VALY MENKIN *From the Ignaz Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia, Pennsylvania*. In numerous earlier studies the writer has demonstrated in many inflammatory types of exudates a pseudoglobulin (cataphoretic studies show it to be in alpha globulin) which is capable of causing the specific growth of granulocytes in the bone marrow and to some extent of megakaryocytes. Besides this growth effect the substance is capable of inducing a discharge of immature polymorphonuclear leukocytes into the circulation, thus offering an explanation for the mechanism of leukocytosis with inflammation. This leukocytosis-promoting factor has been abbreviated as the LPF. The LPF can be preserved for several weeks *in vacuo* under phosphoric anhydride. After such an interval the factor tends to lose its biological potency. Gradually some chemical change occurs, presumably a spontaneous denaturation occurs. After several months the factor loses its initial solubility in an aqueous medium, and it generally becomes either very weak in its activity or else it becomes completely inactive. As shown previously, in such form the LPF can still be recovered as a soluble polypeptide which splits from the rest of the inactive molecule. The remaining portion of the inactive molecule of the original but now presumably denaturated LPF contains a leukopenic factor, and this explains the relative biological inactivity of the aged LPF. The leukopenic component counteracts the leukocytosis-promoting one. Both components can however be fairly well separated from each other by centrifugation and thus studied as individual entities.

**Hypoplastic anemia induced in guinea pigs by 4-amino pteroyl glutamic acid** VIRGINIA MINNICH (by invitation) and CARL V. MOORE *Dept of Internal Medicine, Washington University School of Med, St. Louis, Mo*. Two folic acid inhibitors, a crude preparation and methyl folic acid, have been reported to produce anemia and leucopenia in chicks, mice, rats, and pigs. These changes have been prevented or reversed by folic

acid. Of considerable interest, therefore, is the observation described in this report that another antagonist of folic acid, crystalline 4-amino pteroyl glutamic acid, induces similar changes in the peripheral blood of guinea pigs which cannot be prevented by either folic acid or liver extracts. Guinea pigs fed a stock ration were given daily by subcutaneous injection 0.5 to 5.0 mg 4-amino pteroyl glutamic acid. They all lost weight and with few exceptions died in 11 to 28 days. Some of the animals developed a profound normocytic anemia and all a leucopenia (1000 to 750 WBC per cu mm). Half of the pigs had an agranulocytosis and half became thrombocytopenic. Varying degrees of marrow hypoplasia were found. If administration of the inhibitor was stopped after the hematologic changes had become profound, a few of the animals showed a striking reticulocytosis, rapid rise in all the cellular elements of the blood, and recovered completely. Highly purified liver extracts given with the inhibitor in doses of 1.5 to 5 USP units intramuscularly per day did not prevent the anemia or leucopenia. Folic acid in amounts 25 to 100 times that of the 4-amino pteroyl glutamic acid and injected simultaneously seemed to prevent the development of leucopenia or thrombocytopenia but not the anemia. Erythrocyte levels as low as 800,000 per cu mm were observed. Preliminary observations on other animals suggest that there may be considerable species difference both in the changes produced by this inhibitor and in the ability of folic acid to prevent them.

**Purification of a substance from bovine serum which reduces the prothrombin time of aged plasma** F. L. MUNRO and MURIEL PLATT MUNRO (introduced by F. R. MILLER) *Charlotte Drake Cardeza Foundation, Department of Medicine, Jefferson Medical College and Hospital, Philadelphia, Pa*. Bovine serum contains relatively large amounts of a substance which reduces the prothrombin time (one stage method) of aged plasma. This substance has been named prothrombin A and "labile" factor by Quick (*Am J Physiol* 150, 405 (1947)). It is possibly the "factor V" described by Owren in "The Coagulation of Blood" Thesis, J. Chr. Gunderson, Oslo 1947, and the accelerating globulin described by Ware, Guest and Seegers (*Science* 106, 41 (1947)). By adsorption on aluminum hydroxide followed by suitable elution in an alkaline medium, this substance may be obtained largely freed from other serum proteins. Since serum does not contain prothrombin or fibrinogen these substances are not present in the eluate as they are in similar eluates prepared from plasma. Calcium ions are essential for the stability of this substance in solution.

**Adrenal cortical atrophy and liver damage**

produced in dogs by feeding 2,2-bis-(parachlorophenyl)-1,1-dichloroethane (DDD) ARTHUR A NELSON and GEORGE A WOODWARD (by invitation) *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D C* DDD is closely related to DDT and has considerable importance as an insecticide Eleven dogs were fed DDD dissolved in corn oil in capsules at levels of 50 to 200 (usually 50 or 50) mg /kg /day for periods of 1 to 21 months Six dogs died, 2 were sacrificed because of poor condition, and 1 was sacrificed while still in apparently good condition after 21 months Two dogs survive, and will continue to be fed DDD Gross and microscopic examination of the tissues of all 9 dogs sacrificed or found dead showed in every one a high degree of adrenal cortical atrophy There was also high grade liver damage consisting chiefly of fatty degeneration and to a lesser extent of atrophy, necrosis and cirrhosis In microscopic sections the adrenal cortical parenchyma was one third to one-half its usual thickness, with much distortion of the normal architecture and alteration of the normal cellular appearances The medulla appeared unaffected Rats and a monkey fed DDD did not show the adrenal cortical atrophy, nor did dogs when fed the very closely related DDT Of some dozens of compounds fed to 300 of our dogs, none except DDD has caused adrenal cortical atrophy even though several have caused severe liver damage, few have affected the adrenal in any way The effect of DDD on the dog adrenal is a striking example of chemical specificity in the causation of organ damage

**Hereditary osteopetrosis of the rabbit,** LOUISE PEARCE *The Rockefeller Institute for Medical Research, Princeton, N J* An hereditary disease of the rabbit greatly resembling, if not identical with, the juvenile form of osteopetrosis of man has occurred in a rabbit breeding colony The condition is inherited as an autosomal recessive character It is present at birth and may be identified by the delayed and imperfect development of the teeth and by the characteristic x-ray appearance of the entire skeleton The shadows are very dense and homogeneous with a lack of structural detail Hydrocephalus is occasionally seen Growth and development are retarded and beginning at about 2 weeks of age, progressive malnutrition with eventual cachexia develops A hypochromic anemia which often reaches severe proportions is usually found The condition is invariably lethal, the average survival being 4 to 5 weeks The bones are hard but brittle, spontaneous fractures, however, are rare In x-ray photographs of older cases, the ends of the ribs and of the long bones are frequently clubbed, the long bones and the femur in particular are shortened and the central portions of the bone shadows show translucent areas In

the younger cases, there is an excessive amount of abnormal spongy bone, the marrow cavities are markedly reduced and the marrow tissue is scanty In older animals, the cavities are larger and contain more abundant marrow together with conspicuous amounts of abnormal fibrous tissue Compared with normal litter mates, serum calcium was lower, serum phosphorus was lower in younger but higher in older cases and serum phosphatase was higher, the blood sugar content was lower and the blood cholesterol values were higher

**The influence of lipid ingestion on the thymol turbidity test** HANS POPPER, FREDERICK STEIGMANN, ALVIN DUBIN (by invitation) and HATTIE M DYNIWICZ (by invitation) *From the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois* MacLagan's thymol turbidity test serves as a valuable indicator of liver cell damage A pathologic increase is less marked when liver cell damage is due to extrahepatic biliary obstruction The turbidity depends upon relationship between lipo-proteins and globulins fractions Extraction with ether but not with petrol ether reduces markedly thymol turbidity in serum, ovalated and heparinized plasma In 12 cases the turbidity in ovalated plasma and serum was identical but markedly reduced in heparinized plasma Dicumarol had no effect Adding heparin to 25 sera reduced turbidity proportionally to the amount added These findings explained by an influence upon serum phospholipids prompted study of the effect of ingestion of various fats upon thymol turbidity Usually 50 gm butter produced an equal or higher rise than 4 egg yolks, 50 gm cholesterol, 30 gm lecithin or 30 cc cod liver oil within 3 or 6 hours It disappeared after 24 hours The butter effect was markedly enhanced by simultaneous administration of choline (11 cases) Choline alone had a lesser influence upon thymol turbidity Butter and choline raised the turbidity significantly in 23 hospital controls (average 2.6 units) In liver diseases (25 cases), especially in cirrhosis and obstructive jaundice, and in gastrointestinal disturbances (10 cases) the rise was much lower as expressed in units and even more so when expressed in percentage of the fasting turbidity Further studies should elucidate the clinical value of the rise in thymol turbidity after butter and choline administration as simple test of fat absorption and the influence of choline upon intestinal absorption of fat

**Peroxidase in the lymphocytes of man in acute inflammation** JOHN W REBUCK and ELIZABETH A MONAGHAN (introduced by F W HARTMAN) *Dept of Anatomy, Univ of Minnesota, Minneapolis and Dept Labs, Henry Ford Hospital, Detroit, Mich* The cytology of the exudate in acute inflammation in man has been studied with an original technical procedure in which serial preparations

are obtained chronologically from a single lesion and are stained like blood smears. The epithelium is scraped away from a small area on the forearm. After inoculation with a suitable inflammatory excitant (egg-white) the lesion is covered by a cover-slip. Cells of the inflammatory exudate migrate to the under surface of the cover slip. When this has been accomplished (30 minutes to 1 hour) the cover-slip is removed, air-dried, and stained with Washburn's method for peroxidase. At the same time, another cover slip is placed over the same lesion and the process is repeated at timed intervals throughout the cycle of inflammation. Transformation of the lymphocytes of man into macrophages proceeds in an orderly fashion in such a simple cycle of inflammation. It is accomplished by numerous gradual functional and structural changes in the lymphocytes as such. One of these changes is assumption of the positive peroxidase reaction by lymphocytic cytoplasm. By 9.5 hours, most of the lymphocytes in the area of inflammation contain numerous peroxidase positive granules diffusely distributed throughout the cell bodies. The lymphocytes are actively phagocytic when this stage is reached. Sometimes it is apparent that a phagocytosed neutrophilic cytoplasmic bud within the lymphocytic cytoplasm has contributed a circumscribed area of peroxidase-positive granules to the cytoplasm of the lymphocyte (exogenous source). An occasional lymphocyte lacks such granules.

**Effect of blood and oxygen on P. knowlesi infection in monkeys.** R. H. RIGDON, *Department of Pathology, School of Medicine, University of Texas, Galveston, Texas*. Previous experimental studies with *P. lophiurae* infection in ducks have shown that anoxemia is a significant factor during the course of the disease. Furthermore, life can be prolonged by placing these moribund birds in an oxygen chamber (*Jour. Lab. and Clin. Med.* 32: 57, 1917). These observations were repeated with *P. knowlesi* infection in monkeys. A total of 27 *Macacus rhesus* animals was used. Temporary clinical improvement occurred when the infected monkeys were put into an oxygen chamber with a concentration of 40-60% oxygen. Malarial infected monkeys when given intraperitoneal injections of human blood likewise showed clinical improvement. These observations would suggest that anoxemia is a significant factor in *P. knowlesi* infection in the monkeys. The destruction of the red cells by the plasmodia produces the anemic anoxia.

**Arteriosclerotic lesions in pyridoxine deficient monkeys.** JAMES F. RINEHART and LOUIS D. GREENBLUM (by invitation), *Division of Pathology, University of California Medical School, San Francisco, California*. Tissues of 4 rhesus monkeys subjected to prolonged pyridoxine deficiency have

been examined. The diet has been previously described (*Fed. Proc.* 5: 222, 1916). Significant arteriosclerotic lesions have been found in 3 of the 4 animals. In 3 animals subjected to pyridoxine deficiency for 5 to 7 months, 2 showed intimal fibrosis chiefly in the small arteries at the renal hilus. One animal subjected to pyridoxine deficiency for 14 months, showed also striking lesions in the coronary arteries. In this animal a broad layer of intimal edema and connective tissue proliferation was seen in major coronary arteries. This animal, in addition, presented an enlarged cirrhotic liver. These lesions may be related to the defect in tryptophane metabolism in the pyridoxine deficient monkey recorded elsewhere in this journal (Greenberg and Rinehart). The preliminary report is made because of its potential significance in the important problem of arteriosclerosis in man. Spontaneous arteriosclerotic lesions have not been encountered in the rhesus monkey and experimental production of arteriosclerosis in this species has not been recorded before.

**The influence of folic acid on hemorrhage anemia in dogs.** F. S. ROBSCHLIT-ROBBINS, *Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester 7, New York*. The influence of folic acid on hemoglobin regeneration has been studied in experimental anemia due to blood removal in dogs. Folic acid was tested in daily doses of 5, 10, 20 and 40 mg. as supplements to lextron with and without iron. These materials were added to a basal ration of salmon bread and fed for a two week period. Net hemoglobin output was determined. Results vary. In some experiments a possible inhibition of hemoglobin production was evident when folic acid was administered. Other experiments demonstrate a negative effect.

**Production in vitro from protein and other solutions of substances resembling "natural" agglutinins and precipitins.** EDWARD C. ROSENOW, *From the Rare Metals Institute of the California Institute of Technology, Pasadena, California, and the Longview Hospital, Cincinnati, Ohio*. In experiments on the *in vitro* production of antibody with heat and  $H_2O_2$ , the yield from broth cultures and filtrates was greater than that from comparable suspensions and filtrates in NaCl solution, suggesting that constituents of the culture medium might be a source of antibody. The effect of keeping at room temperature for 24 hours and autoclaving for one hour protein and other solutions before and after adding 1.5%  $H_2O_2$  and 10,000,000,000 pneumococci and streptococci aa per ml from dense suspensions in glycerol two parts and saturated NaCl solution one part was studied in parallel from the standpoint of antibody production. Agglutinins and precipitins for streptococci, pneumococci



and staphylococci were not formed at room temperature in 24 hours in the absence of  $H_2O_2$  and either were not formed or were formed in minimal titer after adding  $H_2O_2$  and 10,000,000,000 streptococci and pneumococci  $\bar{m}$  per ml to each of 5% solutions in NaCl solution of gelatin, peptone, potato starch, gum arabic, essential amino acids, 10% egg white and white and egg yolk  $\bar{m}$ , dextrose brain broth, undiluted milk, and isotonic NaCl solution. Significant but variable titers were obtained from each of these solutions on autoclaving for one hour after adding 1.5%  $H_2O_2$ , and in all instances titers were far greater especially in the case of casein and amino acids on autoclaving after adding both  $H_2O_2$  and the bacteria. The agglutinins produced in the protein and NaCl solution suspensions were filtrable, dialyzable and distillable before conjugation but were no longer dialyzable or distillable after conjugation onto normal globulin.

**Preparation of radioactive gold colloids for use in the therapy of malignancies.** GEORGE ROUSSEAU (introduced by P. F. HAHN) *Vanderbilt University School of Med., Nashville, Tenn.* The use of radioactive gold colloids in the therapy of malignancies made a reliable method for producing them desirable. A procedure was devised in which the pH, rate and order of addition of reagents and temperature were controlled to give a stable colloidal gold solution of uniform particle size for various concentrations of gold. Colloids as concentrated as 5 mg/ml were prepared with a uniform particle size using gelatine as a supporting colloid. The influence of pH, rate and order of addition of reagents and temperature is more pronounced as the concentration of gold is increased. The colloids were prepared by dissolving metallic gold in aqua regia, evaporation to dryness at 100°C under reduced pressure, solution of the auric chloride in water and its reduction to a metallic gold colloid with ascorbic acid using gelatine as a supporting colloid. It was found that better control of particle size could be achieved by the slow addition of an aqueous solution of the auric chloride (chloraureic acid) to an aqueous solution of ascorbic acid and gelatine. The latter solution can then be heated to any desired temperature and the pH adjusted to any desired value by the addition of hydrochloric acid, sodium hydroxide or a suitable buffer without the danger of exposure to intense radiations.

**Structure and repair of the olfactory mucosa in rhesus monkeys.** EDWIN W. SCHULTZ *Department of Bacteriology and Experimental Pathology, School of Medicine, Stanford University, Calif.* Observations were made on normal and zinc sulfate treated mucosae. These were removed *in toto* and in a way which served to preserve their normal relationships to surrounding structures. They were fixed in Bouin's solution, embedded in

paraffin and stained by a modification of the Bodian method of staining for neurofibrils. The normal mucosae were examined for variations in general structure, in the number and distribution of the olfactory cells, and for differences in the structure of the dendritic processes. Zinc sulfate solution constitutes a highly satisfactory agent for destroying the olfactory epithelium in regeneration studies. It induces a massive coagulation necrosis which is followed by a clean *en masse* separation of the necrotic epithelium from the underlying lamina propria. Stages of repair ranging from the 2nd day to the 8th month were observed. Interest centered primarily around the origin of the new epithelium, and the possible source of varying numbers of olfactory nerve cells present after the twelfth day.

**Hydrolysis of DL-leucylglycylglycine by sera of tuberculous and normal rabbits.** JULIUS SCHULTZ (by invitation) and CHARLES WEISS *Laboratories of the Jewish Hospital, Philadelphia, 41, Pa.* The rate of hydrolysis of the tripeptide, DL-leucylglycylglycine (LGG), by aminopeptidase found in sera of tuberculous rabbits was compared with that of normal sera. Seven rabbits were injected intratracheally with 0.00095 mg of a virulent Ravenel culture of tubercle bacilli. From 42 to 71 days later their sera were tested at least twice at intervals of about 10 days or more (in most cases). The average of 19 analyses was  $0.41 \pm 0.03\%$  hydrolysis per minute at a concentration of 0.1 ml serum per ml test solution. The average rate for seven control rabbits, carried out simultaneously, was  $0.184 \pm 0.012\%$  hydrolysis per minute at the same serum concentration. This represents more than a twofold increase in the rate of enzyme activity. These data are significant in the light of the work of Emmart and Seibert (*J. Immunol.* 50:143, 1945) who showed that the globulin fraction of tuberculous serum is tuberculostatic and of the finding of Bruton (*J. Biol. Chem.* 166:721, 1946) that this protein fraction contains the LGG splitting peptidase. Since Weiss and Halliday (*J. Immunol.* 49:251, 1944) have previously observed that extracts of Cathepsin II, obtained from tissues more resistant to tuberculous infection, hydrolyses BA1 faster than that obtained from less resistant organs, the present findings lend further support to the hypothesis that increased proteolytic activity is associated with increased resistance to infection in tuberculosis. The extent to which these results are peculiar to tuberculosis will be investigated.

**Further studies on the hepatic potentiation of the estrogenic activity of triphenylchloroethylene.** ALBERT SEGALOFF and RICHARD L. CORPEDGE (by invitation) *From the Departments of Medicine and Physiology, Tulane University and the Alton Ochsner Medical Foundation, New Orleans,*

*Louisana* We have previously shown that less triphenylchloroethylene is required to produce vaginal estrus in spayed female rats when given intrasplenically than when given subcutaneously. This was interpreted as indicating that the liver increased the estrogenic activity of this compound. We have also demonstrated that more  $\alpha$  estradiol is required to produce vaginal estrus by intrasplenic injection than by subcutaneous injection. This was interpreted as indicating that  $\alpha$ -estradiol was inactivated by the liver. When these studies with  $\alpha$  estradiol were repeated in partially hepatectomized animals, the amount required to produce vaginal estrus was greatly reduced, indicating further that the  $\alpha$  estradiol was inactivated by the liver. Accordingly triphenylchloroethylene was injected into partially hepatectomized female rats by the same two routes. There was increased potentiation of the estrogenic activity rather than the expected decrease. In the attempt to clarify this apparently anomalous result *in vitro* studies were undertaken. It was found that when an amount of triphenylchloroethylene below threshold for increasing the uterine weight of immature female rats was incubated with either liver slices or brei, then significant uterine weight increases were obtained. There is an optimal amount of brei which produces a greater potentiation than larger or smaller amounts of brei. Little or no potentiation of estrogenic activity is obtained with either very small or very large amounts of liver brei. It thus appears that there are two processes in the hepatic metabolism of triphenylchloroethylene: first the conversion to a more active compound, and then the inactivation of this product by oxidation or conjugation.

**Chemotherapeutic effect of chloromycetin on experimental infection with psittacosis and lymphogranuloma venereum viruses** JOSEPH E. SMADFL and ELIZABETH B. JACKSON (by invitation) *Department of Virus and Rickettsial Diseases, Army Medical Department Research and Graduate School, Army Medical Center, Washington 12, D. C.* Chloromycetin, a new antibiotic, has previously been reported to possess chemotherapeutic activity against a number of experimental rickettsial infections. Growth in embryonated eggs of strains of psittacosis virus of pigeon or psittacine origin and of lymphogranuloma venereum virus is reduced by treatment with Chloromycetin. Administration of the drug 48 hours after the infection is almost as effective in prolonging the life of embryos as the prophylactic treatment. Doses of 0.0625 mg. per egg produce a distinct prolongation of life in infected eggs. Furthermore, the length of survival is directly proportional to the dose of drug over the range from 0.0625 mg. to 0.5 mg. per egg. Mice infected by the

intra-peritoneal route with the 6 BC strain of psittacosis survive 80 minimal lethal doses when given 0.75 mg. to 2.5 mg. of drug per day either by mouth or intra-peritoneally. In addition, treatment is effective in mice when delayed for 6 days after infection, i. e., several days before death would be expected to occur. Mice infected by the intra-cerebral route with the P-1 strain of psittacosis and with lymphogranuloma venereum virus are benefited little if at all by treatment with Chloromycetin administered either by mouth or intra-peritoneally. Data bearing on the explanation for the failure to obtain a chemotherapeutic effect following intracerebral infection with these viruses will be presented.

**Experimental hypervolemia, time of onset and some associated physiological and chemical changes** HARRY SOBELL (by invitation) and JACOB FURTH *Cornell University Medical College, New York City and Veterans Administration Hospital—Southwestern Medical College, Dallas 2, Texas* Repeated blood volume determinations were made by the Evans Blue technique on mice bearing transplanted granulosa cell tumors. The blood volume began to rise after the tumors had reached a moderate size. Once this process was set in motion the blood volume increased rapidly and consistently from values below 10% of body weight to values as high as 28%, in the present series. This hypervolemia is not accompanied by an increase in the extracellular fluid (thiocyanate space). The plasma protein values may be normal and when they are reduced this is due to a decrease of plasma globulin concentration. The absolute amount of plasma albumin is, however, greatly increased and this increase roughly parallels the degree of hypervolemia. The blood urea levels may be high or normal in the presence of advanced hypervolemia. Other values will be given and the theory of hypervolemia discussed.

**Production of arteriosclerosis in dogs with cholesterol and thiouracil** ALFRED STEINER, MARGARET BEVANS, and FORREST E. KENDALL (introduced by H. P. SMITH) *From the Research Service, First (Columbia) Division, Goldwater Memorial Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University* The experimental production of intimal arteriosclerosis by the feeding of cholesterol to dogs whose thyroid function was depressed by thiouracil (Steiner and Kendall Arch. Path. 42: 133, 1946) has been repeated in two young dogs. The dogs, which were 4 months old at the beginning of the experiment, were maintained upon a diet of prepared dog food. 10 grams of cholesterol, dissolved in ether was added to the diet each day, the ether was evaporated off and the diet was moistened with milk before feeding. From 0.5 to 1.2 gms. of

thiouracil was administered each day. No additional fat was fed. This regimen resulted in serum cholesterol levels averaging above 1000 mg per cent throughout the experiment. The animals were autopsied after 12 months. Extensive arteriosclerotic lesions developed in the aorta, in the coronary, mesenteric, renal and thyroid arteries and in the arteries of the upper and lower extremities. Of special interest were well defined lesions of the large cerebral arteries.

**Desoxypyridoxine —Morphologic and functional changes in acute pyridoxine deficiency.** HERBERT C. STOEHR (introduced by Dr. A. M. PAPPENHEIMER, SR.) *Dept. of Bacteriology, Harvard Medical School, Boston, Mass.* The administration of desoxypyridoxine to mice and rats produced marked atrophy of normal and of neoplastic lymphoid tissue and impairment of antibody responses. These changes failed to occur when pyridoxine-HCl was given together with the analogue. The severity of the manifestations of the disease, beginning from effective levels, was independent of the dosage of the pyridoxine antagonist. The loss of lymphoid tissue was not mediated by adrenal cortical hyperactivity, since adrenalectomy did not modify the extent of lymphoid atrophy in acute pyridoxine deficiency. Following the administration of desoxypyridoxine, lymphosarcoma transplants showed marked regression associated with extensive pyknosis and caryorrhexis of tumor lymphocytes and apparent transformation of tumor cells into multinucleated giant cells. Rats treated with desoxypyridoxine showed marked impairment of immune responses. The anamnestic response, in mice and rats, was abolished in acute pyridoxine deficiency, whereas it was found unimpaired in adrenalectomized mice. The rate of disappearance of rat antibodies in rats (passive immunization) was not accelerated by the deficiency. Tyzzer's disease occurred in many instances in mice treated with desoxypyridoxine. This infection had never before been observed in the strain of mice employed.

**Some factors associated with variations in nitrogen and ash content of bone.** LOUIS J. STROBINO (introduced by LEE E. FARR) *Alfred I. du Pont Institute of the Nemours Foundation, Wilmington, Delaware.* Reports in the literature indicate considerable variation in nitrogen content of bone. The present study sought to determine some of the factors responsible in beef and human bones. Samples from the interepiphyseal cortex of long bones carefully cleaned of extraneous material were dried to constant weight and analyzed for nitrogen and ash. Nitrogen was determined by the gasometric micro Kjeldahl method of Van Slyke and ash was estimated gravimetrically after heating in a muffle furnace at 600°C. Serial samples were obtained longitudinally between the

epiphyses and circumferentially at various levels of the bone shaft. In addition, subperiosteal bone samples were compared with adjacent samples contiguous to the endosteum. In young subject's bones, an area of high ash, low nitrogen content was observed with a regular increase in nitrogen content and corresponding decrease in ash content as the epiphyses were approached. The position in the shaft of the area of maximum ash-nitrogen ratio was found to be characteristic of the bone. With increasing age, longitudinal variations in ash-nitrogen ratio of the shaft gradually diminished so that by the sixth decade of life, the ash-nitrogen ratio was approximately constant and was somewhat greater than the maximum ratio found in bones of young subjects. Analyses of beef bone were found to correspond to those of humans. When the subject's age and the geometric position of the samples are considered, there becomes apparent a reasonable constancy in nitrogen and ash content of different bones.

**Prolonged parenteral plasma produces hyperproteinemia and proteinuria in dogs and maintains nitrogen equilibrium and health.** ROGER TERRI (introduced by G. H. WHIPPLE) *School of Medicine and Dentistry, University of Rochester, Department of Pathology.* In seven separate experiments five normal dogs received homologous heparinized plasma parenterally as a sole source of nitrogen for periods of 15-92 days. If the period of plasma administration is preceded by a fairly long period of protein starvation, there is a strong positive nitrogen balance for the first 7-10 days of plasma injections and nitrogen equilibrium is maintained thereafter. No excessive excretion of nitrogen is noted in the after period. If adequate calories and vitamins are supplied, weight balance is maintained and the injected plasma seems to be somewhat better utilized. Blood plasma concentrations rise rapidly and vary from 9.5-11.5 gms % during the period of plasma administration. Soon after the hyperproteinemia is noted, proteinuria occurs in measureable amounts. All protein fractions except fibrinogen are present in the bladder urine and are apparently excreted at a constant rate. Globulins form about 30% of the urinary protein. After plasma injections are stopped and after the blood plasma concentration falls below 9 gms %, the proteinuria rapidly diminishes and ceases entirely, indicating no permanent kidney damage. These experiments confirm the concept of a "dynamic equilibrium" between the plasma proteins and tissue proteins. (One dog was maintained in health and nitrogen equilibrium for 3 months with plasma as a sole source of nitrogen.) They also suggest (1) a "ceiling" of utilization of plasma protein, and (2) a renal threshold for protein molecules, in that proteinuria occurs when the circulating plasma

concentration is maintained at levels greater than 9-10 gms %

**Specificity of human serum antihyaluronidase for antagonism of a particular species of bacterial hyaluronidase** ROBERT T THOMPSON and FRANCES E MOSES (introduced by M A BLANKENHORN) *Departments of Internal Medicine and Biochemistry, College of Medicine, University of Cincinnati* McClean's demonstration of the antigenicity of hyaluronidases in rabbits (1913) indicates that an antihyaluronidase serum is specifically antagonistic to the particular species of bacterial hyaluronidase which was used as the antigen. The present study investigates this principle in the sera of humans who suffered bacteremia due to various bacteria. A modification of the mucoprotein clot prevention (MCP) test (McClean) was used to study the sera of eight patients. Serial sera of three patients with pneumococcus bacteremia, one with staphylococcus bacteremia, one with *B. coli* Communis bacteremia, and one with *B. typhosus* bacteremia were titrated for antihyaluronidase against each of three hyaluronidases from culture filtrates of pneumococcus, staphylococcus, and *Cl. welchii*. Sera of the three patients with pneumococcus bacteremia exhibited eight-fold increases of antagonism to pneumococcus hyaluronidase, but no increase of antagonism to staphylococcus and *Cl. welchii* hyaluronidases. Sera of the patient with staphylococcus bacteremia exhibited an eight fold increase of antagonism to staphylococcus hyaluronidase, but no significant increase of antagonism to pneumococcus and *Cl. welchii* hyaluronidases. Sera of the patients with *B. coli* and *B. typhosus* bacteremia exhibited no increase of antagonism to any of the three hyaluronidases. Serial sera of two other patients with pneumococcus bacteremia and empyema exhibited increases of antagonism to pneumococcus hyaluronidase, but no antagonism

to *Cl. welchii* hyaluronidase. It has been reported previously that this human serum antagonism to pneumococcus hyaluronidase is not specific for type of pneumococcus.

**Further studies on a fat-soluble biotin-active material (FSF) from plasma** WILLIAM TRAGER *Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.* Experiments in addition to those previously reported have again shown that the oil obtained from hydrolyzed horse plasma has biotin-like activity for chickens as well as for bacteria. When chickens fed a diet high in egg-white were injected intramuscularly with this oil in the maximum dosage which could be well tolerated (equivalent by microbiological assay with *Lactobacillus casei* to 1 microgram of biotin per week), they developed less dermatitis of the mouth and feet than did uninjected chickens on the same diet. The effect was almost as good as that obtained with the injection of 1 microgram of biotin itself. Chickens similarly injected with oleic acid showed as severe a dermatitis as the untreated controls. Hence, although oleic acid has about 3 times the biotin activity for *L. casei* of the oil from horse plasma, it cannot account for the biotin like effect of the latter material in chickens. Fractionation of the oil from horse plasma has yielded preparations which are probably not pure but which on a weight basis have as high a biotin activity for *L. casei* as oleic acid. These preparations are solid, whitish, waxy materials at room temperatures. Their activity in chickens has not yet been tested. FSF activity (the biotin activity liberated by acid autoclaving and removed by ether from alkaline suspension) is much higher in the plasma of egg-laying chickens and ducks than in the plasma of males or non-egg laying female birds of comparable age.

## AMERICAN INSTITUTE OF NUTRITION

## TWELTH ANNUAL MEETING

*Atlantic City, New Jersey, March 15, 16, 17, 18, 19, 1948*

(For possible corrections in any of the following abstracts see the next issue)

**Studies on reproduction and lactation in rats and mice maintained on synthetic diets** SISTER ANN MIRIAM ALLGLIER (by invitation), ALBERT J. SICA (by invitation), LEONORA MIROVNE (by invitation), FRANK P. PANZARELLA (by invitation), and LEOPOLD R. CERECEDO *Department of Biochemistry, Fordham University, New York* Experiments on reproduction and lactation in rats and mice maintained on synthetic diets have shown the following facts: 1. There is a close connection between birth-weight in the rat, nutrition of the mother before and during pregnancy in the rat and the mouse, and survival of the young. 2. The first four days in the life of the mouse and the first three days in the life of the rat are critical periods. Non-survival during these periods points to inadequacy for reproduction of the diet received by the mother. Such failures fall into the same category as stillbirths, resorptions and abortions. 3. In all studies on reproduction and lactation, the previous nutritional history of the animals must be considered. Our experiments with rats have shown that pteroylglutamic acid, given only during pregnancy and lactation, has some beneficial effect. However, a more pronounced effect on both reproduction and lactation was obtained when the supplement was given from weaning. The relative merits of so-called "long-term" and "short-term" experiments in the study of the nutritional requirements for reproduction and lactation will be discussed.

**Effect of arsonic acid derivatives in stimulating growth of chicks fed certain diets** H. R. BIRD, A. C. GROSCHKE (by invitation), and MAX RUBIN (by invitation) *The growth of chickens fed diets high in soybean meal and deficient in the unknown dietary factor found in fish meal and in cow manure was improved by the addition to the diet of 0.005% of 3-nitro-4-hydroxyphenyl arsonic acid. The effect of higher or lower levels was less favorable. This compound did not function as a substitute either for the unknown factor or for methionine. On the contrary, with diets containing raw soybeans, the arsonic acid derivative, the unknown factor, and methionine were mutually supplementary. Such a supplementary effect could not be demonstrated with diets containing commercially heated soybean meal because addition of the unknown factor induced essentially optimal growth in the absence of other supplements. The arsonic acid was effective when fed with 35% or with 70% of commercially heated soybean meal, but less effective than the unknown factor. Other com-*

pounds comparable in their activity to 3-nitro-4-hydroxyphenyl arsonic acid were phenyl arsonic acid, p-hydroxyphenyl arsonic acid, and m-nitrophenyl arsonic acid. The following were tested and found inactive: p-chlorophenyl arsonic acid, sodium arsenate, m-nitrobenzenesulfonic acid, salicylic acid, 3-nitrosalicylic acid, and sodium p-phenolsulfonate.

**Protein requirements of ten women and adequacy of estimated quantities for ten weeks** MILDRED L. BRICKER (by invitation), RUTH F. SHRIVELL (by invitation), and JANICE M. SMITH *Agricultural Experiment Station, Dept. of Home Economics, University of Illinois, Urbana, Illinois* Ten girls, 19-30 years of age, cooperated in this study of nitrogen balances which lasted for 105 days. Four intakes of nitrogen (approximately 1.8, 3.1, 0.2, and 4.3-6.7 grams daily) were ingested during four periods. The first two periods of ten days each and the third low nitrogen period of 15 days duration were used in estimating the minimum amount of a protein mixture required for nitrogen equilibrium. During the fourth period, 70 days in length, the adequacy of the calculated protein requirement was tested for each of the subjects by continuous nitrogen balance data, weekly hemoglobin determinations, red cell counts and performance tests. The protein mixture, composed of bread, rolled oats, lean beef, light cream and white potatoes in proportions to furnish 59.5, 10.5, 14.7, 13.4 and 1.9% of the protein intake respectively, had an average nutritive index (biological value  $\times$  true digestibility) of 60.9. The quantities of protein of this composition needed for nitrogen equilibrium plus the amounts calculated to be needed for "adult growth" were found to be 31.7, 35.2, 27.1, 30.2, 30.9, 26.9, 29.4, 42.2 and 35.2 grams daily for each of nine subjects. Throughout the ten-week balance period, nine of the subjects remained in definite positive nitrogen balance, hemoglobin and red cell values were maintained at initial levels and performance tests including critical fusion frequency of flicker, speed of tapping, endurance on a bicycle ergometer and mental and emotional responses showed no deterioration of function.

**Niacin and protein relationships in corn diets for growing pigs** W. T. BURNETT (by invitation), R. C. MILLER, and R. A. DUTCHER *Pennsylvania State College, State College, Pennsylvania* Young growing pigs were fed high corn diets containing additional protein from either casein or corn gluten

Diets containing 15 and 20 per cent of protein were compared. In addition all of the pigs received thiamine, riboflavin, pyridoxine, pantothenic acid, inositol, p-aminobenzoic acid, cirotene, vitamin D and a complete salt mixture. Characteristic symptoms of niacin deficiency did not occur with pigs fed combinations of corn and corn gluten at either the 15 or the 20 per cent levels of protein intake, or with pigs fed the 20 per cent protein diet containing 5 per cent of casein. Pigs on the 15 per cent protein diet, containing 5 per cent of casein, grew more slowly unless given supplemental niacin. Niacin was without any apparent effect on growth on the exclusive corn diets at either protein level. The N'-methylnicotinamide excretion indicated increased methylation with the niacin supplemented pigs, except in the case of those pigs receiving the 20 per cent protein diet containing 5 per cent of casein. Further increased methylation occurred when both choline and niacin were added to the diet simultaneously. However, the feeding of niacin and choline in combination resulted in an unfavorable growth response. There was little if any correlation between N'-methylnicotinamide excretion and growth response in these studies.

**Achromotrichia produced in mice on a cooked egg diet** EDITH M. CARLISLE (by invitation) and HELEN T. PARSONS, *Dept. of Home Economics, University of Wisconsin, Madison*. The hair of black mice of a non cancerous strain, C<sub>57</sub>, turned an all over silver gray on a diet of egg white and yolk cooked with salts and later cerelose added. These mice developed low hemoglobin levels and showed marked edema. Another group of mice were fed a similar ration except the yolks and whites were cooked separately and the mineral salts added later. These mice showed little or no change in the color of their original coat of hair and maintained normal hemoglobin levels. The addition of copper to the ration of the strikingly gray mice stimulated a rapid growth of thick black hair clearly observed in areas of skin after depilation. Their hemoglobin values rose rapidly. Hence the cause of the grayness was attributable to a relative unavailability of the copper of the ration. It has been shown in other laboratories that a deficiency of copper produces in the rabbit an achromotrichia which seems to appear before the anemia, and also that the copper of egg yolk is relatively unavailable to the rat. These present experiments show that copper undergoes a change when cooked with egg which renders this mineral unavailable to the mouse.

**Changes in the water content of the albino rat during the first week of dietary rehabilitation** ESTHER DA COSTA (introduced by R. E. JOHNSON) and RUTH CLAYTON (introduced by R. E. JOHNSON). With the technical assistance of Corporal GARY G. LALANNE, *U. S. Army Medical Nutrition*

*Laboratory, Chicago 9, Illinois*. After 13 weeks of dietary restriction, i.e., (a) low calorie, (b) with 1% protein from carrots, and (c) with 10% special salt and 5% protein, 8 rats from each of these groups and controls were sacrificed and the water content of the total carcasses determined. Twenty-four animals from each group were rehabilitated on diets isocaloric with the control diet but in experiment one containing 60% protein, and in experiment two, 35% fat. In experiment one the water content of the carcasses of the controls was 64%. The water content of the carcasses of the refed low calorie rats was 68.8% after 24 hrs., 65.7% after 48 hrs., 65.5% after 72 hrs. and 67% after 96 hrs. The refed carrot diet rats showed 68.6%, 68.5%, 68.1% and 65.7% water content after 24, 48, 72 and 96 hrs. of refeding. The refed high salt animals had a water content of 68.8%, 69.3%, 67.7% and 66.5% in the same units of time. During the 13 preliminary weeks, the controls gained 32%, the restricted animals lost between 21 and 28%. In one week of rehabilitation, the refed animal gained from 26 to 37%. In experiment two the changes in water content and weight during restriction were of the same order as in experiment one but during rehabilitation water was lost more rapidly and weight gained more rapidly than in experiment one.

Thus in one week of feeding a high protein diet after restriction there was a gradual dehydration, and on a high fat diet a rapid dehydration.

**Studies on the cellulose growth factor for chicks** FRANK DAVIS (by invitation) and GEORGE M. BRIGGS, *Department of Poultry Husbandry, University of Maryland, College Park, Maryland*. Davis and Briggs (*J. Nutrition* 34: 293, 1947) reported the growth-promoting action of cellulose in purified chick diets. Using the same experimental procedure and basal ration, degradation products and substances associated with and structurally similar to cellulose were tested for their effect on chick growth. Mixed sawdust composed of white and yellow pine, levulinic acid and D-xylose were fed separately to day old New Hampshire chicks at various supplemental levels at the expense of glucose in the ration. Statistical analysis of chick weights at the end of repeated 4 week trials showed that the growth increments obtained with 0.1% of levulinic acid and 10% of mixed sawdust were highly significant. Supplementation with 0.05 through 2% of D-xylose gave no appreciable growth response. Preliminary experiments with furfural and D-cellobiose indicate that these compounds possess little or no cellulose growth activity. Furfural, however, at the 0.1 and 0.3% supplemental levels gave a growth response similar to 0.1% of levulinic acid. These observations indicate that 10% of mixed sawdust, 0.1% of levulinic acid, and 0.1 through 0.3% of furfural can

probably be substituted for 5% of cellulose in chick purified diets with equal growth response

**Pteroylglutamic acid balance studies on monkeys** PAUL L. DAY, DOROTHY S. GAINES (by invitation), MARION MCKEE (by invitation), PHYLIS SCROGIN (by invitation), and RAYMOND HOLCHINS (by invitation) *Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock* Juvenile rhesus monkeys were given a diet known to produce the anemia and leucopenia of pteroylglutamic acid deficiency, supplemented with the amounts of pteroylglutamic acid (PGA) or related materials as indicated below, for periods of 21 days or more. Daily urine and feces collections were made. Assays for free PGA were made on the urine, and for free and conjugated PGA on the feces, using *Streptococcus faecalis* and a suitable conjugase. When the diet was supplemented with 100 micrograms of PGA daily, the average daily output in micrograms was as follows: urine, 19; feces, 5.8; total, 10.7. When the daily dietary supplement of PGA was increased to 1000 micrograms the output in micrograms was: urine, 21.0; feces, 9.1; total 30.1. In another experiment the following supplements were made to the diets of comparable monkeys: 2.5 grams Difco yeast extract containing approximately 100 micrograms of PGA (largely as conjugase), 100 micrograms of PGA, 100 micrograms of pterioic acid, negative control receiving no supplement. The outputs of PGA in urine and feces of these animals were essentially the same. In summary, a ten fold increase in PGA intake resulted in a four-fold increase in urinary output, but did not appreciably alter the fecal output of the vitamin. Monkeys receiving microbiologically equivalent amounts of PGA, PGA conjugate (as yeast extract), or pterioic acid, and a negative control, all excreted equivalent amounts of microbiologically active material in the urine and in the feces.

**Changes in dehydro and total ascorbic acid of cantaloupes on standing** KATHERINE J. ELLIOTT (by invitation), SHIH DZUNG CHEN, (by invitation) and CECILIA SCHUCK *Purdue University* In the past the original ascorbic content of foods and losses on standing have usually been reported in terms of reduced ascorbic acid only. It is now questionable as to whether reported losses of ascorbic, when only the reduced form has been determined, represent true losses. Recent studies have shown that decreases in the reduced ascorbic acid of cabbage could be accounted for largely by conversion to dehydro ascorbic acid (*Science* 103, 196 (1946), *Journal American Dietetic Association* 23, 223 (1947). The present study was carried out to determine the amount of dehydro, reduced and total ascorbic acid in cantaloupes immediately after cutting and after standing in the refrigerator and at room temperature for 24 hours. The melons were

cut crosswise through the center with a plastic knife and a circle removed for analysis. Half of the remainder of each melon was then placed in the refrigerator and half allowed to stand at room temperature. Both halves were wrapped in waxed paper. The diphenylhydrazine procedure of Roe and Oesterling, (*Journal Biological Chemistry* 152, 511, (1944)), was used to determine the dehydro and total ascorbic acid and the reduced ascorbic acid was obtained by difference. The findings indicate that there was partial conversion of reduced ascorbic acid to the dehydro form both in the refrigerated melons and those held at room temperature, the change being somewhat greater in those held at room temperature. The results suggest a need for the determination of total and dehydro ascorbic acid in other foods under different conditions of handling and preparation.

**Influence of liver extracts on a sulfathiazole induced dietary deficiency in rats** GLADYS A. EMERSON and CHARLES W. MUSHETT (by invitation) *Merck Institute for Therapeutic Research, Rahway, N. J.* A number of sulfonamides have been fed to rats as adjuncts to purified diets containing all identified vitamins except pteroylglutamic acid. A deficiency state evidenced by a failure in growth and a dyscrasia was noted after 4-5 weeks on test. Animals maintained on the ration containing 1% sulfathiazole made a rapid response to the administration of certain (4 out of 5) parenteral liver extracts as did those receiving adequate dosage of pteroylglutamic acid. Since the content of pteroylglutamic acid or its conjugates in the liver preparations was insufficient to account for the curative action, the presence of an unknown factor is indicated. The parenteral extract which was without effect in these tests was active in the treatment of the deficiency state induced by the feeding of a low protein diet (Daft and Sebrell). A limited number of experiments have been conducted with mice maintained on rations containing 1.25% sulfathiazole. This species also responded to a liver extract.

**Anemia in chronic choline deficiency in the rat** R. W. ENGEL (introduced by W. D. SALMON) *Laby of Animal Nutrition, Alabama Polytechnic Inst., Auburn* Weanling rats of the Alabama Agricultural Experiment Station (AES) strain developed an anemia in 2 to 3 months when fed diets deficient in choline or substitute nutrients. The anemia was characterized by a reduction in hemoglobin to as low as 5 gm. per 100 ml. blood, a reduction in total red blood cells to as low as 3.5 million per cmm., and a lowered color index. The severity of this condition was directly related to the degree of deficiency of the diet in choline or substitute nutrients. The diets employed ranged in composition from 4.5% to 18% in extracted casein, from 20% to 40% in degerminated corn

grits or alcohol extracted peanut meal, and from 2 to 20% in lard. All diets contained 4% salt mixture and sucrose to make 100%. The diets were fortified with adequate amounts of thiamine, riboflavin, pyridoxin, niacin, inositol, ca pantothenate, a-tocopherol, and vitamins A and D in the form of cod liver oil. The anemia was prevented by fortifying the diets with choline Cl (2 gm/kg) or with DL-methionine (3 gm/kg). The sodium salt of pteroyl di glutamic acid (1 mg/kg) was ineffective as a curative agent when added to a choline-deficient diet.

**Occurrence of B-vitamins in tissues of rats fed rations satisfactory and unsatisfactory for reproduction.** GLADYS EVERSON (by invitation), ELEANOR WILLIAMS (by invitation), ELIZABETH WHEELER (by invitation), PEARL SWANSON, MARGARET EPPRIGHT (by invitation) and MARTIE SPIVEY (by invitation) *The Nutrition Laboratory, The Foods and Nutrition Section, Iowa Agricultural Experiment Station, Iowa State College, Ames*. Toxemia of pregnancy in addition to other manifestations of unsatisfactory reproduction, i.e., sterility, resorptions and production of non-viable young have been encountered in female rats receiving a ration containing partially dried autoclaved pork muscle and five parts of yeast as the main sources of protein and the B-vitamins. In an effort to determine the dietary cause of these unsuccessful reproductions, assays have been made of the respective concentrations of thiamine, riboflavin, nicotinic acid, biotin, pantothenic acid, and folic acid in the liver, carcass, and fetuses of pregnant rats fed the ration. For comparison, similar assays have been conducted on tissues of animals receiving a modified Steenbock stock ration adequate for the support of normal reproduction. Vitamin stores were studied throughout two pregnancies. Analyses showed that the two rations provided widely differing amounts of pantothenic acid and biotin, the pork containing diet being less rich in these factors than the stock ration. The hepatic tissues of rats ingesting the lower intakes contained smaller quantities of both biotin and pantothenic acid than did those of the control animals. The concentration of the two vitamins in the fetal tissues of the pork-fed rats also was less than that in the young of females successfully undergoing reproduction. The occurrence of all six B-vitamins in hepatic, carcass, and fetal tissue will be given for animals receiving both types of rations.

**The pantothenate requirements of the mouse, with observations on the role of biotin, inositol, and p-aminobenzoic acid.** PAUL F. FENTON and GEORGE R. COWGILL *Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University*. Male mice of the C57, C3H and A strains were placed on a pantothenate deficient basal diet for one week. After this depletion they were fed

the identical basal diet with graded doses of pantothenic acid. At all levels of pantothenate studied the C57 strain mice grew better than those of the C3H and A strains. Deficiency symptoms (achromotrichia, alopecia, dry dermatitis) appeared early in the C57 mice on low intakes of pantothenate. C3H and A strain mice, on the other hand, showed only occasional slight alopecia. The blood picture (red and white cell counts and hemoglobin levels) of C3H and A strain mice remained essentially within normal limits. C57 mice on low pantothenate diets showed a lowering of the red cell count and hemoglobin levels, while occasional animals showed abnormally high white cell counts. The pantothenate levels in muscle and liver were determined microbiologically. Biotin injections were found to speed the restoration of hair and the return of normal color. A large group of C57, A and I strain mice were placed on one of four synthetic rations: a) complete, b) deficient in inositol, c) lacking p-aminobenzoic acid, and d) without inositol or p-aminobenzoic acid. No clear cut symptoms of inositol or p-aminobenzoic acid deficiency could be demonstrated. Another group was fed these same diets after being depleted of pantothenic acid. A fourth group received a diet moderately low in pantothenate and devoid of p-aminobenzoic acid and inositol. Studies of the cecal flora were made.

**Thiouracil content of poultry tissue following prolonged feeding of the compound.** A. L. FRANKLIN (by invitation), J. W. BOEHM III (by invitation) and T. H. JUKES *Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.* Numerous studies indicate that the feeding of thiouracil to poultry improves carcass grade and increases feeding efficiency. Information is lacking, however, regarding the extent to which thiouracil is stored in the tissues, and information regarding this point is necessary before thiouracil may be fed to chickens which are intended for human consumption. Satisfactory chemical methods for the determination of thiouracil in tissues are not available. A biochemical method was used in which the tissue thiouracil content was ascertained by determining the degree of depression of thyroid function in rats which had been fed the dried tissue for a three week period at a 50% level in the diet. This was accomplished by measuring the accumulation of an injected dose of radioactive iodine and the formation of thyroid hormone from this iodine by the thyroid gland. After feeding thiouracil at a 0.2% level to chickens for 3 weeks the thiouracil content of the muscular tissue was found to be  $3 \pm 2$  mg %. When the thiouracil supplementation was discontinued for 24 and 48 hours the tissue thiouracil content fell to about 0.1 mg % and less than 0.05 mg % respectively. Cooking the meat for one hour at 130° had no effect on the level of thiouracil, however storage of the



tissue at 4° for one week resulted in the disappearance of most of the compound from the tissue

The utilization of nicotinic acid by pregnant women ERNESTINE I FRAZIER (by invitation), THILMA PORTER and MARY JANE HUMPHREY (by invitation) *Dept of Home Economics, Univ of Chicago* The intake of nicotinic acid and the urinary excretion of its acid hydrolyzable products and N<sup>1</sup> methylnicotinamide of 7 primiparas on self selected diets was studied for 8 day periods during each of the last 5 months of pregnancy. The average daily intakes of 6 of the primiparas ranged from 12.8 to 20 milligrams. The seventh subject had an average intake of 33.7 milligrams per day including 20 milligrams of nicotinamide. On intakes up to 20 milligrams there was an increase in the excretion of N<sup>1</sup> methylnicotinamide during the last trimester of pregnancy. The level of excretion of the subject on the higher intake remained quite constant through the eighth month after which there was a decrease in excretion of the methyl derivative. The average daily excretion of N<sup>1</sup>-methylnicotinamide for all subjects ranged from 13.0 to 16.2 milligrams from the fifth through the ninth month of pregnancy. The level of excretion of the acid hydrolyzable fraction showed only slight variations with a range of average daily excretion of 0.76 milligrams on an average intake of 12.8 to 15.7 milligrams on the average intake of 33.7. On an average intake of 12.2 milligrams of nicotinic acid per day the average daily excretion of N<sup>1</sup>-methylnicotinamide for 44 clinic patients was 13.3 milligrams. When grouped according to months of pregnancy, the same general trend in increased excretion was noted. There was considerable variation in percentage return of test dose as N<sup>1</sup> methylnicotinamide, irrespective of daily intake, with a trend toward increased return with increased duration of pregnancy.

The absorption of iron from beef by women RUTH FRENCHMAN, EUPHEMIA D BOROLGHS, FRANCES A JOHNSON (introduced by HAZEL M HAUCK) *N Y State College of Home Economics, Cornell University, Ithaca, N Y* Five young women 18 years of age and in good health, with normal serum iron, hemoglobin and red cell counts, were placed on a diet containing 7 mg of iron which was judged to be the lowest intake that would be adequate. After six weeks on the basal diet, two 100 gm patties of ground beef ( $\frac{3}{4}$  lean meat,  $\frac{1}{4}$  fat) containing 3.4 mg of iron were added, one at breakfast and one at lunch. The increase in retention after the addition of the beef was considered as iron absorbed from the beef. The absorption of iron from beef for four out of five subjects were 32, 45, 58 and 57 per cent. This led to the conclusion that young women 18 years of age on an adequate diet absorb from about one-third to more than one half of the iron of beef. Another interpretation

of the results may be the correct one that the beef improved the absorption of the iron of the entire food mixture, rather than itself contributing all of the iron absorbed after it was added to the diet. If the amount of iron added to the food residue as it passes through the gastro intestinal tract is negligible, the mean absorption from the basal diet was 11 per cent and from the basal diet plus beef was 21 per cent.

The turbidimetric determination of infused fat in blood after intravenous administration of fat emulsions ROBERT P GEYER (by invitation), GEORGE V MANN (by invitation), and FREDRICK J STARE *Dept of Nutrition, Harvard School of Public Health and Dept of Biological Chemistry, Harvard Medical School, Boston* A turbidimetric method for the determination of the rate of disappearance of infused fat from the blood has been developed. After the intravenous administration of a fat emulsion, 20 cmm samples of blood are withdrawn at intervals from the animal and are added to 5% dextrose solution. Following centrifugation at slow speed, the supernatant is decanted and to it are added concentrated ammonium hydroxide (to remove extraneous turbidity) and hydrogen peroxide (to remove hemoglobin). The turbidity which remains after heating to 60°C is determined by means of a photoelectric colorimeter using a 420 mμ filter. Since the optical density is a linear function of the concentration, the latter can be obtained from a standard curve. Results obtained by the use of this method agree well with those obtained by the use of either the micro-oxidative or the chylomicronograph technique. The term "fat tolerance curve" is used to designate the curve which results when the concentration of fat in the blood is plotted against time. Such curves have been determined for the rat, dog, and rabbit after the administration of an emulsion containing 30% coconut oil and 3% phosphatides. The first two species remove the fat at rapid rates, while the rabbit does so more slowly. When mineral oil was substituted for the coconut oil in the case of the rabbit, the oil was removed from the blood at a very slow rate.

Minimal protein requirement for growth in the rat MARIANNE GOERTSCH *Department of Chemistry, School of Tropical Medicine, San Juan, Puerto Rico* The growth and reproduction of rats was observed on diets ranging from 7.1 to 19.1 per cent protein, supplied by polished rice, 56.4 parts, red kidney beans, 28.2, and casein, 15.4. Minerals, vitamins and cornstarch were added. The diets contained 1.5 per cent fat and 3.4 Calories (calculated) per g. The "true digestibility" and biological value of the N were determined by appropriate metabolism tests on rats to be respectively 84.1 and 74.7. Diets containing 16.7 or greater per cent of protein supported growth in several genera-

tions of rats that was equal to that of controls on an adequate diet. Rats receiving 14.3 per cent protein from the 21st day, grew approximately as well as controls, but in successive generations, they grew less rapidly. With further reduction in dietary protein, the rate of growth decreased in rats given the diets from the 21st day. Upon 7.1 per cent protein there appeared the hepatic necrosis associated with methionine deficiency. The maximum value for the coefficient of protein utilization for growth, as determined by Osborne and Mendel, occurred on diets containing 14.3, 11.9, and 9.5 per cent protein and was 2.30. This is further proof that the 14.3 per cent level was inadequate. The 16.7 per cent diet contained 7.8 mg T N per Calorie. Experiments are in progress to determine whether (1) Approximately 50 mg net T N per Calorie of any protein is the minimum for growth (2) The coefficient of utilization of the most efficient protein, under these conditions, is approximately 3.70.

**Urinary excretion of B vitamins in persons on normal and restricted diets.** GRACE A. GOLDSMITH and HERBERT P. SARETT. *Nutrition Research Laboratory, Tulane University School of Medicine, New Orleans.* In eight normal persons on adequate diets of varied composition the urinary excretion of thiamine in 24 hours was over 100  $\mu$ g, of riboflavin over 500  $\mu$ g, and of N'-Methylnicotinamide (N'-Me) over 3.5 mg. In 4 hours after a test dose of thiamine (5 mg), riboflavin (5 mg) and niacinamide (50 mg) mean thiamine excretion was 240  $\mu$ g, riboflavin 2.3 mg, and N'-Me 6.4 mg. In seven normal persons on weighed diets providing 2500 calories, 40 gm protein, 0.7 mg thiamine, 0.5 mg riboflavin and 6 mg niacin, the urinary excretion of B vitamins decreased to relatively constant values, which averaged 19  $\mu$ g of thiamine, 56  $\mu$ g of riboflavin and 1.4 mg of N'-Me. After three to six weeks on this diet the average four hour excretion in response to a test dose was 58  $\mu$ g of thiamine and 1.3 mg of riboflavin. Excretion of N'-Me is not included in this report since the basal diet was supplemented with tryptophane before testing. In three patients with beriberi, thiamine excretion was 13 to 55  $\mu$ g in 24 hours and 21 to 55  $\mu$ g after a test dose. Of ten patients with B complex deficiency (glossitis, diarrhea, cheilosis, macrocytic anemia) two showed a thiamine excretion of less than 100  $\mu$ g in 24 hours and four an output of less than 60  $\mu$ g after a test dose. Riboflavin excretion in 24 hours was extremely variable (270 to 1800  $\mu$ g) and could not be correlated with test dose findings in appraising nutritional status. In 3 of the subjects excretion of N'-Me was less than 3 mg in 24 hours and less than 3.5 mg after a test dose. Nutritional evaluation based on excretion levels will be discussed.

**The effect of threonine in choline deficiency**

WINDLI H. GRIFFITH and MARY F. NAWROCKI (by invitation). *Department of Biological Chemistry, St. Louis University, School of Medicine.* The severity of renal hemorrhagic degeneration and the extent of deposition of liver fat are markedly increased in weanling rats by the addition of threonine to a low-choline, cystine supplemented 8 per cent casein diet. No other essential amino acid shows a similar effect. Threonine is ineffective in the absence of supplementary cystine. The influence of threonine is not due to a direct antagonistic action on choline or on labile methyl but is the result of a stimulation of growth or metabolism which aggravates the existing choline deficiency. The effect of threonine is analogous to the previously demonstrated role of supplementary cystine in diets low in total sulfur amino acids (Mulford, D. J., and Griffith, W. H., *J. Nutrition*, 23:91 (1942)). This finding supports the conclusion that threonine and cystine are the most important limiting amino acids in casein fed at an 8 per cent level. If the 8 per cent casein diet is supplemented with cystine and threonine and with sufficient choline to permit survival, then other amino acids show apparent antilipotropic action. This experimental method provides a rapid and simple procedure for the determination of the priority of limiting amino acids in proteins. Application of the procedure to the determination of the relative metabolic activity of isomers and of derivatives of amino acids and to the recognition of suboptimal intakes of vitamins and of other nutrients is in progress.

**The effects of folic acid on respiratory metabolism.** R. C. GRUBBS (by invitation), B. C. HOUGHTON (by invitation), JULIA TROSSBACH (by invitation) and F. A. HITCHCOCK. *The Laboratory of Aviation Physiology, The Ohio State University, Columbus.* Studies on respiratory metabolism and nitrogen balance before and during the administration of folic acid have been carried out on hospital patients amenable to folic acid and on normal controls. Oxygen consumption, carbon dioxide production and non-protein respiratory quotients were determined using the Tissot-Haldane technique under the following conditions: basal, one hour after a standard meal and before, during and after walking on a treadmill at a slow rate. All subjects lived in the hospital and were on a weighed diet. A two or three week control period was followed by a two or three week period during which 30 mgm. of folic acid were administered intramuscularly daily. Results indicate no significant change in basal oxygen consumption. There was an average increase of 11 per cent in the calories derived from carbohydrate in normals and of 6.5 per cent in patients. There was an increase in specific dynamic action in all patients and in approximately one half of the normal subjects. In the nitrogen balance

studies, adequate diets were given and the caloric intake and nitrogen of the diet kept constant. An aliquot portion of the food was prepared each day for analysis. All urine and stools during the balance period were collected and total nitrogen determined by the Kjeldahl method. Folic acid stimulated the appetite and increased nitrogen retention of patients but produced no consistent effect on the normals.

**Factors affecting the excretion of metabolites of phenylalanine and tyrosine in alkaptonuria** W. K. KOWALTON HALL (by invitation), V. P. SIDENSTRICKER and KATHLEEN RAWLS (by invitation) *Depts. of Medicine and of Biochemistry, Univ. of Georgia School of Medicine, Augusta*. One alkaptonuric adult female from a group of seven females and four males, all alkaptonuric and all related, was hospitalized for metabolic studies. The subject was fed a diet containing 70 gm. of protein per day and the urinary excretion of homogentisic acid, total nitrogen, organic acids, acetone bodies and creatinine were followed for a two week period and later for a three day period. The homogentisic acid excretion and homogentisic acid to nitrogen ratios were somewhat variable and were not significantly affected by the administration of an autolyzed yeast preparation, various types of oral and parenteral liver extracts or by six days on a ketogenic diet. As had been previously determined with other subjects with alkaptonuria from this group, phenolic compounds other than homogentisic acid were excreted in the urine. One or more of these compounds were precursors of melanin since on standing, 0.4 gm. or more of melanin per 24 hour specimen would form in the acidified urine, though there was no change in the homogentisic acid content. The total phenol rather than the homogentisic acid excretion roughly corresponded to the probable phenylalanine and tyrosine content of the diet. Similar results were obtained in a three day study of urine samples from a five year old alkaptonuric boy, apparently unrelated to the other alkaptonuric individuals. Studies were also made of factors influencing the excretion of homogentisic acid by rats fed a high tyrosine diet.

**Dietary factors in experimental renal hypertension I** **Protein** PHILIP HANDLER and F. BERNHEIM (by invitation) *Departments of Biochemistry and of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.* Series 1. Adult male rats, eating a commercial chow were rendered hypertensive by the Raska technic. Three weeks later, when systolic pressures were stabilized at 145-160 mm., they were divided into 3 groups of 12 rats each which were fed chow (A) and synthetic rations containing 50 (B) and 5% (C) casein. Pressures in group A remained at 155 mm. for 4 weeks and rose to 190-210 mm. before

death (mean survival 51 days). Pressures in group B rose to 185-240 mm. within 1 week and all animals died within 4 weeks (mean 37 days). Pressures in group C fell to 95-130 mm. within 3 weeks, remained at that level for 2-5 weeks and then rose before death (mean survival 63 days). Series 2. Rats were fed synthetic rations containing 50 (D), 25 (E) and 5% (F) casein for 2 weeks before surgery and continued on these diets thereafter. Two weeks later mean systolic pressures were D 168 mm., E 141 mm., and F 114 mm. Pressures in group D rose to 180-220 mm. for 3 weeks and fell to subnormal levels in the last week as the animals lost weight. Pressures in group F remained low for 4 weeks and rose to 180-210 mm. the last week accompanied by a drastic weight loss. Group E remained at 140-155 mm. for 4 weeks and then behaved like group F. No correlation was observed between pressure and blood NPN or protein concentration. Studies are now in progress of the effects of 10% casein, choline deficiency, urea feeding, specific amino acids, and of salt feeding and deprivation on high and low protein diets.

**Susceptibility to infection manifested by dogs on a low fat diet** ARILD E. HANSEN, OLETA BECK (by invitation) and HILDA F. WIESE (by invitation) *Dept. of Pediatrics, Univ. of Texas Medical School, Galveston*. Over a period of 4 years, it has been observed that young dogs maintained on a diet low in fat show an apparent lowered resistance to infection, especially of the skin. Of a total of 16 animals on the fat deficient diet, 9 have shown superficial impetigo-like infections of the skin. These have been resistant to external treatment. The skin of these animals also showed the desquamation characteristic for dogs on the low fat diet, whereas control animals receiving fresh lard in the diet quite uniformly manifest a healthy skin. The fat deficient animals become markedly emaciated and seem to be susceptible to pneumonia. Three of these dogs died and showed evidence of extensive pulmonary consolidation on autopsy. None of the control animals housed under identical conditions have shown any susceptibility to pneumonia. Especially significant has been the involvement of the ears in 10 animals. There has been a continuous purulent discharge from their ears. This condition has never developed in the control dogs. *Pseudomonas aeruginosa*, *alcaligenes fecalis*, paracolon and streptococci have been cultured from the purulent discharges. A striking cessation of the exudate from the infected ears occurs when fat is added to the diet. This has occurred when lard, bacon fat or butter fat have been substituted isocalorically for sucrose in the diet. Gain in weight, and a generally improved reaction of the animal together with the appearance of new healthy skin are also manifested to a greater or

lesser degree depending on the kind of fat which is added to the diet

**The effect of diet upon iron absorption** D MARK HEGSTED, CLLEMENT A FINCH (by invitation), and THOMAS D KINNEY (by invitation) *Department of Nutrition, Harvard School of Public Health, the Departments of Biological Chemistry, Medicine, and Pathology, Harvard Medical School, and the Medical Clinic and Department of Pathology, Peter Bent Brigham Hospital, Boston* Modern studies on the absorption of iron from the intestinal tract have indicated that the amount absorbed is dependent upon the body needs for iron (Hahn, et al, *J Exp Med* 78 169, 1913) and the work of Granick (*J Biol Chem* 161 737, 1916) has supported the possibility that the control is by ferritin which acts as a block to iron absorption. However, the studies of Gillman, Mandelstam, and Gillman (*S Afr J Med Sci* 10 109, 1915) and the work to be presented show that the absorption of iron is greatly influenced by the diet. Gillman et al found a large proportion of South Africans to have excessive amounts of iron in their livers. We have shown that rats fed a diet composed chiefly of corn grits supplemented with iron deposit 10 to 20 times the normal amount of iron in the liver within a period of 4 to 6 weeks. Rats receiving adequate diets containing the same amount of iron show normal liver iron values. The excessive iron deposition is demonstrable histologically in amounts which parallel the chemically determined values and is found chiefly within the parenchymal liver cells. Storable iron is also found in a few other organs when the liver values are high. The effect of various dietary supplements in preventing excessive iron absorption has been studied. None of the vitamins appeared to be active. Protein supplementation was only slightly effective while a complete salt mixture was more effective. Phosphate salts alone were similarly active but did not completely prevent a rise in liver iron.

**Dental caries in the rat, mus norvegicus** JULIA O HOLMES, L R Parkinson (by invitation), ANNE W WERTZ (by invitation), and LOIS BROWN (by invitation) *Agricultural Experiment Station, University of Massachusetts, Amherst, Massachusetts* The study of tooth decay in the Albino rat has been continued, two strains of rats being used. The rations have been fed ad libitum for 14 weeks and have contained casein 15 to 21%, mineral mixture 4, fraction L liver powder 1, corn oil 1, an adequate vitamin mixture, and either sucrose, glucose, dextrin or starch as the carbohydrate. Areas of yellow or opaque enamel or of yellow dentin, particularly soft areas, and decayed areas have been recorded as carious and the extent of decay has been scored on a scale of 1-2-3. On rations containing fermentable carbohydrates the average scores for incidence and extent of decay

were 10 and 13, respectively, in strain I, and 19 and 36 in strain II. On diets containing starch or dextrin decay was less, the scores averaging 5 and 6 for strain I and 11 and 14 for strain II. Using basal rations of the above composition, several foods or biological compounds have been assayed for their caries-preventing potency. Urea prevented tooth decay. Some of the supplements increased the caries scores. The lowering of the casein level from 20 to 7% doubled the incidence and tripled the extent of decay. The data indicate that sugars are not the only components of the diet which may be conducive to tooth decay.

**$\alpha$ -Tocopherol and certain nitrogenous compounds as factors influencing the mortality of rats after CCl<sub>4</sub> poisoning** E L HOVE *Research Laboratories, Distillation Products, Inc., Rochester, New York* Vitamin L and certain nitrogenous compounds have been found to influence the susceptibility of rats to carbon tetrachloride poisoning. The basal diet contained casein (10%), sucrose (76%), lard (10%), salt mixture, and an adequacy of pure vitamins, except vitamin E. Carbon tetrachloride was given intraperitoneally at a level of 2 cc per kilogram body weight to groups of rats on this diet, with or without various supplements. The mortality of groups of rats on the basal diet was 90 to 100% within 18 hours after poisoning. Alpha-tocopherol (1 mg daily) reduced the mortality to less than 20%. Supplements given for six weeks but stopped at the time of poisoning afforded several days protection but did not prevent ultimate death. Continuation of the supplements after poisoning gave continued protection. The activity of  $\alpha$ -tocopherol was obliterated by 6-methylthiourea (0.1%), which had no activity by itself. Gamma-tocopherol was only slightly active at a level of 1 mg daily. The protective action of  $\alpha$ -tocopherol given to rats in the vitamin L free basal diet could be duplicated by methionine, xanthine derivatives, or other purines, or by an increased casein level in the diet. The influence of several individual amino acids has also been investigated. This technique is of value for the study of the intermediary metabolism of  $\alpha$ -tocopherol and its several interrelations.

**Relative importance of growth and metabolic rate on the utilization of vitamin A by the rat.** R M JOHNSON (by invitation) and C A BAUMANN *Department of Biochemistry, College of Agriculture, Univ of Wisconsin, Madison* Wandering rats were fed sufficient halibut liver oil to produce hepatic stores of approximately 250 gamma of vitamin A. They were then maintained on various low A diets designed to produce different rates of growth or different metabolic rates in the animals. The tissues of the animals were then analyzed at intervals. When growth was normal, the hepatic stores of vitamin A decreased and the concentration of vitamin in the kidney increased.

The rate of decrease from the liver was accelerated slightly when the metabolic rate was high, it was decreased when the metabolic rate was low, e g when thionin was fed. Retention of liver stores was highest when growth was suppressed. In general the rate of utilization of stores vitamin A appeared to depend on both the growth rate and basal metabolic rate, but to a greater degree on the former.

**The adequacy of an intake of seven milligrams of iron per day for women.** FRANCES A. JOHNSTON, EUPHEMIA D. BOROUGHS, RUTH FRENCHMAN (introduced by HAZEL M. HAUCK). *N Y State College of Home Economics, Cornell University, Ithaca, N Y*. Five young women were placed on a diet of 7 mg of iron for a four week period. Seven milligrams was selected because Leverton (J Nutrition 21: 617, 1941) had found retentions were large enough to cover all losses on that intake. In this study, when the amounts of iron lost in urine and stools were considered, retentions were 0.64, 0.26, 0.43, 0.61, 1.33 mg per day. The menstrual losses per period ranged from 10.27 to 28.08 mg. In one case the retention was more than enough to cover menstrual losses and in four cases the retentions almost equalled the losses. Those subjects whose menstrual losses approximated their retentions were probably not obtaining sufficient iron to cover all their needs because no account was taken of iron lost in perspiration, skin, nails and hair. The conclusion drawn from this study was that an intake of seven milligrams of iron was on the borderline of the requirement for four of these five women when the diet was adequate in other respects and contained one small serving of a low iron flesh food. It was below the requirement if the iron content of perspiration is an appreciable amount. When beef containing 3.4 mg of iron was added to the diet, retentions ranged from 1.77 to 2.53 mg per day. This high retention with beef may explain the better retentions found by Leverton than by us on the 7 mg intake, as the diet she used contained much beef.

**The inhibitory effect of excess methionine on protein utilization.** CHARLES F. KADE, JR. and JESSY SHEPHERD (introduced by AARON ARNOLD). *From Biology Division, Sterling Winthrop Research Institute, Rensselaer, N Y*. It is well known that the growth rate of the rat can be increased by the addition of small amounts of methionine to a diet containing suboptimal quantities of casein. Studies were made to determine the effect of supplements of methionine on the growth of rats fed diets containing casein at an intake giving the highest protein efficiency. It was found that a diet containing 8 per cent casein (based on nitrogen  $\times 6.25$ ) permits the greatest utilization of the protein. Varying quantities of DL-methionine up to 1 per cent added to this diet resulted in increasing

protein utilization. An 8 per cent casein diet supplemented with 1.5 per cent DL-methionine gave little better growth than the diet without additional methionine, 2 per cent, 2.5 per cent and 3 per cent added methionine definitely inhibited growth and protein utilization. In order to rule out taste factors and differences in caloric intakes a set of weanling rats were pair fed isocaloric diets differing only in the DL-methionine content. The rats fed the 8 per cent casein diet supplemented with 1.0 per cent DL-methionine grew the best, showing the highest protein efficiency. Two per cent DL-methionine added to casein was little better than the unsupplemented casein. Thus greater than optimum quantities of methionine are of no value in improving the utilization of casein and may actually be inhibitory. In all probability the mixture of amino acids in which the proportion most nearly approaches the quantitative requirements of the animal for the essential amino acids will be the most efficiently utilized.

**Effect of xanthophyll on the utilization of carotene and vitamin A by the rat.** BARBARA KELLEY (by invitation) and HARRY G. DAY. *Dept of Chemistry, Indiana Univ., Bloomington*. Weanling rats were made vitamin A deficient by standard procedures. After growth had nearly ceased they were divided into similar groups as respects weight and sex. In each experiment  $\beta$ -carotene in cottonseed oil was given daily to one group, the amounts ranging from 4 to 20  $\gamma$  per supplement in the different experiments. A second group was given exactly the same amount of carotene plus 20 to 100 times as much xanthophyll (lutein) in equal amounts of cottonseed oil. A third group received xanthophyll alone and the fourth received neither carotene nor xanthophyll. The supplements were given for 21 to 28 days. In all the experiments rats receiving carotene alone gained more weight than those given xanthophyll in addition to the carotene, although the gains were quite variable. The amount of vitamin A in the livers was decidedly greater in the rats given carotene alone. Also, marked differences were found when vitamin A (DPI concentrate) was given as the supplement and relatively large amounts of xanthophyll were added. This was particularly notable with regard to the effect on vitamin A in the kidneys. Rats receiving xanthophyll plus vitamin A had less than one half as much of the vitamin in the kidneys. The results suggest that the effect of non-provitamin A xanthophyll on growth and the vitamin A content of the tissues is not due to a specific impairment of the enzymatic mechanism for the conversion of carotene into vitamin A.

**Biological analysis of rice.** MARINUS C. KIR. *Department of Agricultural Chemistry, University of Arkansas, Fayetteville*. Studies were continued to reveal the losses of vitamins in the regular

practice of commercial rice milling. Two series of rat feeding experiments were initiated to learn whether whole brown rice was a better source of biotin and folic acid than white polished rice. **Biotin** In triad feeding, using 12 triads, no differences in gain were found and no increased growth was obtained from whole brown-rice-rations to which biotin was added. A slightly better growth was obtained on the whole brown-rice-rations in paired feeding tests. **Folic acid** In 12 triads, polished rice was inferior to whole brown rice as a source of folic acid. The brown rice ration was not improved by the addition of more folic acid. No difference was found in paired feeding tests. In these experiments, all rats received daily 20  $\mu$ g of thiamine, 20  $\mu$ g of riboflavin, a 20  $\mu$ g of pyridoxine, 6 mg of choline, 120  $\mu$ g of Ca pantothenate, 10 mg of inositol, 3 mg of para amino benzoic acid, 2.5  $\mu$ g of biotin and 5  $\mu$ g of folic acid. In addition, each animal received 3 drops per week of halibut liver oil as supplementary sources of vitamins A and D. The vitamin to be tested was omitted.

In rats fed white polished rice diets with and without *niacin*, the same growth was obtained. A similar experience was obtained with rats fed brown rice diets. In each case 12 pairs of rats were used in paired feeding experiments. This suggests that white polished rice and whole brown rice, unlike corn or corn grits, do not have any growth retarding effect in rats.

**The nutritive value of cereal proteins in human subjects** CARL A. KUTHLER (by invitation) and VICTOR C. MYERS, *Department of Biochemistry, Western Reserve University, Cleveland*. Nitrogen balances were measured in eight adult human subjects (3 F, 5 M) eating weighed diets composed of cereal, milk, cream, sucrose, butter and vitamin capsules. The diets were designed to provide 0.7 gm of protein ( $N \times 6.25$ ) and approximately 37.5 Cal per kilo per day, with the cereal providing 55, 80 or 100 per cent of the protein. The cereals used were both processed and unprocessed oats, wheat and corn. The nitrogen of the exploded oat cereal (Cheerioats) was utilized as well as the nitrogen of rolled oats (mean replacement value, 101 per cent) under these conditions. Supplementation with DL-lysine improved the nitrogen balance in every case, the extent of the improvement being the same for both of these cereals. Both wheat flakes and exploded wheat were inferior to whole wheat in maintaining nitrogen balance (mean replacement values of 95 and 84 per cent, resp.), indicating damage to the protein in the processing of these cereals. Corn flakes and an expanded corn cereal (Kix) were approximately equal to whole corn in maintaining nitrogen equilibrium (mean replacement values of 99 and 101 per cent, resp.) under the conditions of this experiment. Three of the male subjects were on all of the diets

where the cereal supplied 100 per cent of the protein. The mean daily nitrogen balances for these three subjects, arranged in the order of decreasing nitrogen balance, were as follows: whole wheat, -0.71 gm, exploded oat cereal, -1.17, wheat flakes, -1.19, rolled oats, -1.22, expanded corn cereal, -1.55, whole corn, -1.60, corn flakes, -1.67, exploded wheat, -2.22.

**The quantitative requirement of the rat for magnesium and effects of magnesium deficiency in the rabbit** H. O. KUNLL (by invitation) and P. B. PLARSON, *Dept. of Biochemistry and Nutrition, A & M College of Texas, College Station*. Diets containing different levels of magnesium ranging from 50 to 300 p.p.m. were fed to growing rats. When the magnesium is provided as the sulfate, between 150 p.p.m. and 200 p.p.m. is required to maintain normal blood magnesium values. Slightly lower values may be sufficient to protect the animal against vasodilatation and hyperexcitability. The magnesium in dried wheat plant or as the oxide or carbonate is less efficiently utilized than magnesium sulfate. Weanling rabbits fed a diet deficient in magnesium exhibit within a period of 5 to 6 weeks a syndrome of hyperexcitability, convulsions, hypomagnesemia and retardation of growth. The addition of magnesium to the diet of rabbits that have ceased to grow results in a prompt resumption of growth. The vasodilatation characteristic of a magnesium deficiency in rats was not observed in rabbits. The magnesium content of the whole blood of rabbits on an adequate diet is approximately 1.8 mg % while the level in the plasma is about 1.6 mg %. The plasma magnesium reaches a minimum level of about 1 mg % after six weeks on the deficient diet. The magnesium content of whole blood continues to decline over a longer period of time to a level of approximately 3.4 mg % after ten weeks.

**Urinary thiamine and riboflavin excretion of children during fasting and under conditions of loading test** WANDA I. LAMECK (by invitation), MARGARET N. CORYELL (by invitation), ELIOT F. BEACH and IRL G. MACY, *Research Laboratory, Children's Fund of Michigan*. During a survey of nutritional status in five institutions the urinary excretion of thiamine and riboflavin by 374 children was determined during fasting and for 4 hours following an oral dose of 1 mg of each of these vitamins. Fluorophotometric methods were used for analysis. The accuracy of short period urine collections was verified by simultaneous creatinine and total nitrogen determinations. The data reveal that in children three to seventeen years old, age and size have little effect either on hourly fasting excretion of B vitamins or response to load tests. In most cases boys showed a higher average excretion than girls, perhaps owing to the greater food consumption. Comparison of the fasting and load

test excretion data for 5 institutions reveals a considerable spread in values which is believed to indicate differences in level of tissue saturation with respect to thiamine and riboflavin. Average fasting hourly thiamine excretions varied from 4.3 to 9.7  $\mu\text{g}$  and 4 hour load test outputs ranged from 104 to 189  $\mu\text{g}$ . Corresponding values for riboflavin were 32 to 55, and 273 to 387  $\mu\text{g}$ , respectively. With either vitamin, correlation between fasting and load test values was low. Between spring and fall, average excretion of thiamine rose from 5.7 to 8.3  $\mu\text{g}$  per hour, apparently due in part to seasonal changes in dietary habit. In one group of children a period of dietary improvement was followed by an increase in the average fasting excretion of riboflavin from 39 to 48  $\mu\text{g}$  per hour.

**Diet and dose-response of weanling rats to intravenous alloxan** GEORGE V. MANN (by invitation) and FREDRICK J. STARE, *Department of Nutrition, Harvard School of Public Health, and Department of Biological Chemistry, Harvard Medical School, Boston*. The relationship of the diabetic response in weanling rats of several strains has been determined by measurement of the degree and duration of diabetes in animals after a single intravenous dose of alloxan monohydrate. Blood sugar levels were measured after a 5-hour fast. Urine sugar was determined on 24 hour urine collections. A fasting blood sugar above 150 mg per cent and 24-hour urine glucose above 0.1 gm was considered as an indication of diabetes. A technique has been developed with which animals can be injected rapidly and accurately through a tail vein without anesthesia. The following table indicates the diabetic response with varying dosage levels of alloxan.

*The diabetic response of young rats after intravenous alloxan*

| Dosage        | No. of rats | 48 hour Response | Immediate mortality |
|---------------|-------------|------------------|---------------------|
| mg/kg body wt |             | %                | %                   |
| 30            | 10          | 10               | 0                   |
| 40            | 10          | 40               | 0                   |
| 50            | 9           | 70               | 0                   |
| 60            | 10          | 90               | 0                   |
| 70            | 32          | 97               | 7*                  |

\* Due to toxic effects of alloxan upon liver and kidneys

The fluctuation of incidence of diabetes after a given dose of alloxan has been found to be related to the nature of the diet fed. It has been found that isocaloric substitution of carbohydrate with protein increases the survival rate of the animals. Substitution of 1% sodium chloride for drinking water lowers the survival rate irrespective of the protein level of the diet. A natural diet (Games Dog Meal) with 25% protein allows a higher survival rate than a synthetic diet containing 45% protein.

**Food fermentation as a preventive of furuncu-**

**culosis** J. F. McCLENDON, *Hahnemann Medical College, Phila.* With the development of canning and soft drinks, the eating and drinking of food products preserved by fermentation has decreased whereas the percentage of calories from sugar and bread have increased, especially during the meat shortage. There are mild diabetics whose only symptom is furunculosis. One such was observed for the last 30 years in relation to the effect of diet, insulin, sulfanilamide, sulfathiazole and penicillin. A high fat meat diet was tried for a few years, then a low available sugar diet for 2 years and a low cellulose hemicellulose diet for a number of years. Since it was found that acetate injected into the blood stream is rapidly metabolized and since acetate is produced and absorbed by the 50-litre rumen of a sheep, a 50 litre crock was used in the fermentation of human food. Since the furunculosis was seasonal, i.e. during the summer when outdoor work and fresh fruits and vegetables stimulated the appetite, the experiments were confined chiefly to the removal of sugar from the food by fermentation and the substitution of pickles for part of the bread of the diet. (On a diet containing coarse corn and leafy vegetables considerable fermentation occurs in the gut and it seems probable that thiamin, riboflavin, pantothenic, nicotinic and folic acids and biotin may be synthesized by bacteria in the gut and absorbed from dead bacteria.) This diet shrinks the skin and reduces body weight and prevents furunculosis. Return to a high available carbohydrate diet reverses all these effects.

**Calcium requirements of seven adolescent girls** BEULA V. MCKAY (by invitation), MRS. ELEANOR SMITH (by invitation), and JANICE M. SMITH, *Agricultural Experiment Station, Dept. of Home Economics, University of Illinois, Urbana, Illinois*. Seven girls, 12-13 years of age, selected on the basis of physiological maturity and willingness to cooperate served as subjects. Continuous calcium, nitrogen and phosphorus balance determinations were made for 79 days during the summer of 1946. Four levels of calcium were fed during consecutive 20 day periods. The basal diet, composed of a variety of natural foods, furnished, on the average, 183 mg calcium daily. To this were added homogenized milk and ice cream in quantities to supply the following average amounts of calcium daily: Period I, 1.995; Period II, 2.303; Period III, 0.935; and Period IV, 0.455 grams. The average daily retention for the seven girls was as follows: Period I, 0.420; Period II, 0.463; Period III, 0.224; and Period IV, 0.035 grams. Individual data were used to determine values for utilization of milk calcium and resulting daily calcium requirements according to the technique of Holmes et al. (*J. Nutrition* 31:127, 1946). The percentage utilization of calcium from milk and ice cream was 23.1, 39.0, 40.1, 30.3,

34.1, 40.0 and 51.2 for each of the seven subjects. Four of the girls showed maximum calcium retentions and their calculated daily calcium requirements were 1.009, 1.654, 1.113 and 0.700 grams. The low figure resulted from the observed high utilization value of 51.2%, not from a lowered retention. One of the three remaining girls did not exhibit maximum calcium retention and the other two were doubtful. Nitrogen and phosphorus data will be reported later.

**Effect of arginine on urinary output of 17-ketosteroids in a patient with myotonia atrophica.**  
A. T. MILHORAT, *Depts. of Psychiatry and Medicine, Cornell Univ. Medical College, the Russell Sage Inst. of Pathology and the New York Hospital, New York, N. Y.* A man aged 46 with moderately advanced myotonia atrophica showed generalized muscular wasting and advanced testicular atrophy. The average daily urinary outputs of preformed creatinine and creatine were 0.750 and 0.2000 gm respectively. The BMR was -26 per cent. The concentration of free cholesterol in the blood serum was 183 mg per cent, the level of cholesterol esters was 467. Examination of the semen showed azoospermia. The daily urinary output of 17 Ketosteroids was only 3.66 mg. Delta-tocopherol in dosages of 500 mg daily for a period of 7 weeks induced no demonstrable changes except an increase in androgen output to a level of 5.95 mg. Administration of delta tocopherol was stopped, and L (+) arginine mono hydrochloride was given daily in amounts of 2 gm for 4 days, 6 gm for 6 days, and subsequently in amounts of 12 gm daily. On the 9th day of arginine feeding the urinary output of 17 Ketosteroids was 6.30 mg and on the 21st day the output had increased to 8.72 mg. No effect on output of preformed creatinine and creatine, BMR, or blood cholesterol was observed. When considered together with the observations of Kochakian and Fraenkel-Conrat, Simpson and Evans that testicular and adrenal cortical hormones increase arginase activity, these findings suggest that arginine and arginase are important components of the mechanism regulating production of these hormones.

**Studies of the occurrence of diketo-l-gulonic acid, dehydro-l-ascorbic acid and l-ascorbic acid.**  
MARY B. MILLS (by invitation), CHARLOTTE M. DAMRON (by invitation), and JOSEPH H. ROE, *Department of Biochemistry, School of Medicine, George Washington University, Washington, D. C.* The 2,4-dinitrophenylhydrazine procedure has been made the basis of a method for the determination of diketogulonic acid, dehydroascorbic acid and ascorbic acid in the presence of each other. A difficulty is the extraction of tissues without producing a change in the partition of the three compounds. This is overcome by grinding the tissues under 10 per cent  $\text{SnCl}_4$  in 5 per cent  $\text{HPO}_3$  solu-

tion, then diluting the slurry with 5 per cent  $\text{HPO}_3$  solution until the  $\text{SnCl}_4$  has a final concentration of 0.5 per cent. This method has been applied to the analysis of plant and animal tissues. Diketogulonic acid does not occur in the tissues of the normal guinea pig in amounts determinable by this method. Dehydroascorbic acid has been observed in very small amounts in guinea pig tissues but it is uncertain whether this is a true finding or the result of oxidation of ascorbic acid during extraction. Fresh foods from the market may show the presence of small amounts of dehydroascorbic acid or they may not contain any of this compound. Processed and dehydrated foods usually show the presence of small quantities of diketogulonic acid and considerable amounts of dehydroascorbic acid. The gradual loss of total ascorbic acid and the rate of change in the partition of ascorbic acid, dehydroascorbic acid and diketogulonic acid in kale, orange juice, and potato slurry in the ice box have been studied.

**Inadequacy of thymine as a nutritional factor for reproduction and lactation in the mouse.**  
LEONORA MIRON (by invitation) and LEOPOLD R. CERECEDO, *Department of Biochemistry, Fordham University.* The ability of thymine to substitute for pteroylglutamic acid in reproduction and lactation in the mouse was investigated in this laboratory. This seemed of interest since Spies, T. D. et al. (*Southern Med. J.*, 39: 269, 1946) reported that thymine was beneficial in human anemias. To the basal diet R 5(a) 6 grams of thymine (diet RT 5) were added in lieu of 10 mg of pteroylglutamic acid (diet RS 5(10)). Sixteen females which had been maintained on the basal diet R 5(a) since weaning and which represented three different strains of mice were fed diet RT-5 at mating. The results obtained are summarized below.

| Diet     | Successful gestation | Litters weaned | Litter size | Weaning weight |
|----------|----------------------|----------------|-------------|----------------|
|          | %                    | %              |             | g              |
| R 5(a)   | 61                   | 33             | 5.5         | 7.5            |
| RT 5     | 70                   | 45             | 6.3         | 6.7            |
| RS-5(10) | 82                   | 70             | 6.3         | 8.5            |

The findings indicate that thymine can not substitute for pteroylglutamic acid as a nutritional factor for reproduction and lactation in the mouse when fed during gestation and lactation. Recently Petering, H. G. and Delor (*Science*, 105: 547, 1947) reported the inability of thymine to substitute for pteroylglutamic acid in the rat.

**Studies on fat digestibility, the effect of melting points and dietary calcium and magnesium levels.**  
MARCELT G. MOREHOUSE (by invitation), AMBER LIENG SHAN CHENG (by invitation) and HARRY J. DEUEL, JR., *Univ. of Southern California School*



*of Medicine* Previous studies on fat digestibility (Crockett, M E, and H J Deuel, Jr, *J Nutr*, 33 187, 1947) have shown that with melting point increase above 50°C a decrease in total digestibility occurs with an increase in the per cent of soap in the unabsorbed fat residue. The present investigation is a study of the effect of calcium and magnesium in the diet on the digestibility of natural and synthetic fats of various melting points. The food consumption of female rats maintained on diets containing 15% fat with or without added calcium and magnesium was recorded. The fat digested was measured by subtracting neutral fat, fatty acids and soaps extracted from fecal residues from the fat consumed. Corrections for endogenous fecal fat were made. The digestibility of partially hydrogenated lard (M P 55) decreased from 75 without to 60% with the inclusion of calcium and magnesium. In the latter case a large proportion of the unabsorbed fat appeared as soaps. The digestibility of a blended lard (M P 48) averaged 95% and was independent of the dietary salt levels. However, the digestibility of synthetic triglycerides melting at this level decreased with the addition of the divalent ions. Trilaurin (M P 47) averaged 95% digestibility without and only 71% with the salt addition. The corresponding changes in the digestibility of trimyristin, tripalmitin, and tristearin were 77 to 38, 28 to 14, and 19 to 11 per cents without and with the salts respectively.

**Studies of thiamine deficiency in C3H mice**  
HAROLD P MORRIS, CELIA S DUBNIK, and THELMA B DUNN *National Cancer Institute, USPHS, Bethesda, Md*. The addition of a small amount of thiamine to a diet devoid of thiamine resulted in relative good growth of young mice for a few weeks, followed by a decline in weight, development of clinical symptoms of thiamine deficiency which terminated in death of the animal unless the mice received thiamine supplements. This syndrome has been designated chronic thiamine deficiency. Gross and histological changes were observed in mice subject to this slowly produced thiamine deficiency. The changes were characterized by an absence of spermatogenesis, by muscular degeneration and by hemorrhages in the mid-brain but by an absence of histologic changes in other parts of the nervous system. The relation of enforced food restriction on survival, body weight and other changes observed in chronic thiamine deficiency will be discussed. Some quantitative values for the amount of thiamine required for growth and maintenance of body weight will be given for C3H mice.

**Composition of Central American foods I**  
Honduras HAZEL E MUNSSELL, ROBERT S HARRIS and LOUIS O WILLIAMS (by invitation), with the technical assistance of LOUISE GUILD (by invita-

tion), GERTRUDE NIGHTINGALE (by invitation) and CYNTHIA TROESCHER (by invitation) *Nutritional Biochemistry Laboratories, Massachusetts Institute of Technology*. For the establishment of a sound program of food production and food consumption it is important to know the composition of the indigenous foods which are, or can be, grown in the area. On this basis a long range study of the composition of foods grown in Central America is underway. Specimens are collected and identified by a botanist, samples representative of the edible portion are stabilized and shipped by air express to Cambridge. Whenever possible a record is made of the soil type, annual rainfall, altitude and whether the sample was grown on fertilized land. A comparison of the data from similar samples may give information on the relative importance of genetic and non genetic factors in determining the composition of edible plants. Moisture, nitrogen, ether soluble fraction, crude fiber, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin and total ascorbic acid content are being measured. In the present report the results of analyses of 129 samples of 85 plants collected in Honduras are presented.

**Response of leukopenia and granulocytopenia in sulfathiazole-fed rats to pteroylglutamic acid and to parenteral liver extracts**  
CHARLES W MUSHETT (by invitation) and GLADYS A EMERSON *Merck Institute for Therapeutic Research, Rahway, N J*. Treatment of rats, which had developed leukopenia and granulocytopenia while maintained on a purified diet containing 1% sulfathiazole, with either pteroylglutamic acid or purified liver extracts containing the antipernicious anemia factor resulted in a prompt restoration to normal of the white cell numbers. A significant increase in reticulocytes was observed also. Differential counts on femoral bone marrow of rats treated with the liver preparations revealed a picture similar to that seen in animals given pteroylglutamic acid. In contrast to the untreated rats which had a predominance of erythroid elements in the bone marrow and few or no polymorphonuclear leukocytes, the treated animals had relatively more myeloid cells, including a large number of segmented neutrophils. This resulted in an increased myeloid erythroid ratio. Microbiological assays of the liver extracts failed to show the presence of a sufficient amount of pteroylglutamic acid or its conjugates to account for the hematopoietic response observed. The leukopenia and granulocytopenia found in rats given a purified diet of low protein content, similar to that used by Daft could also be corrected by injections of the highly purified liver extracts.

**Nutrients required by the rat for reproduction and lactation**  
B L O'DELL (by invitation) and A G HOGAN *Department of Agricultural Chem-*

istry, University of Missouri, Columbia Rats from the stock colony were placed on a synthetic ration composed of acid washed casein, cerelese, wood pulp, salts, lard, vitamins A, D, E, and K, thiamine, riboflavin, pyridoxine, and calcium pantothenate All of the surviving offspring were allowed to remain with the dam for four weeks On the basal ration 48 per cent of the young were weaned with an average weight of 42.2 g The weaning percentages and weaning weights resulting from the addition of various vitamin combinations were as follows Niacin, 34%, 37.6 g, choline and niacin, 51%, 48 g, biotin, choline, and niacin, 66%, 46.9 g, pteroylglutamic acid, biotin, choline, niacin and ascorbic acid, 75%, 56.4 g, inositol, pteroylglutamic acid, biotin, choline, niacin and ascorbic acid, 71%, 56.3 g, inositol, biotin, choline, niacin, and ascorbic acid, 62%, 53.2 g When the casein was extracted with alcohol the response was 71%, 52.6 g, when lactalbumin was substituted for casein the response was 66%, 51.0 g In other trials the amount of vitamin A in the diets varied from 100 to 2000 I U per 100 g and the amount of vitamin E from 0.5 to 2.5 mg Of 1628 young rats born to dams receiving pteroylglutamic acid, only 2 or 0.12% developed hydrocephalus Of 2134 young born to dams which did not receive the vitamin, 22 or 1.03% developed hydrocephalus There was no correlation between any of the other dietary modifications mentioned and the incidence of hydrocephalus in the young

**Haemoglobin levels by age and sex** L B PERRY and G F OGILVIE (by invitation) *Nutrition Division, Department of National Health and Welfare, Ottawa, Canada* Although haemoglobin estimations have been used for a century and are important in nutrition surveys, data are difficult to interpret for several reasons (1) methods in common use differ widely in their accuracy, some being quite unsuitable, (2) results expressed in percentages may not be clear since at least 5 different percentage standards are in use, (3) there is no really "normal" value and little information is available on ranges to be expected in a mixed population, especially at different ages In the course of doing nutrition surveys in many parts of Canada, thousands of haemoglobin estimations have been made on people of all ages, using one photoelectric apparatus This paper reports some of the sources of error involved in haemoglobin estimations generally, and the results tabulated by age and sex of 3,148 estimations from age 1 to 79 While the averages found are not suggested as necessarily "normal", yet they represent the most widespread collection of figures in Canada on unselected people, obtained by the same procedure As has been shown before, male haemoglobin levels gradually rise to a peak about age 20, in this report the peak average was 14.5 grams Female levels

showed no such consistent rise with age, although the averages found for different age groups varied from 12.3 grams to 13.2 The use is advocated of distribution curves rather than averages or percentages in recording low haemoglobins for surveys

**The effect of vitamin therapy upon serum blood levels** H B PILGEL, R F KRAUSE (by invitation), J H BROWL (by invitation), SUSAN MERROW (by invitation) with the technical assistance of C A NEWHALL (by invitation), T H HARWOOD (by invitation), HATTIE KAPLAN (by invitation), ANN BAKER (by invitation), JEAN PARKER (by invitation), LORRAINE CARPENTER (by invitation) *Departments of Biochemistry, Medicine and Anatomy, College of Medicine, University of Vermont, Burlington, Vermont* Vitamin therapy has been used for one year with a group of school children having all degrees of several lesions assigned to a deficiency of one or more vitamins Niacinamide, riboflavin, ascorbic acid and vitamin A are being given to treat lesions generally attributed to their respective deficiencies Blood samples have been analyzed three times during the year, in winter, spring and fall, according to the microchemical methods of Bessey and associates Therapy with vitamin C resulted in a rapid and marked increase in serum ascorbic acid levels, however, in vitamin A therapy, serum levels of this vitamin did not rise as rapidly or as high proportionately The percentage of children on A therapy showing increased blood levels of vitamin A was less than that noted for vitamin C in the ascorbic acid group In all therapy groups serum ascorbic acid and vitamin A levels are higher than in control groups regardless of the vitamin used for therapy Control groups continue to show a high percentage of children with low serum values of ascorbic acid, vitamin A and carotene Computations derived from food intakes show that greatest inadequacies in the diet are vitamin A and ascorbic acid

**Pantothenic acid requirement of adrenalectomized rats and its relation to the white blood cells** ELAINE P RALLI, MARY E DUMM (by invitation), and PAUL ROTH (by invitation) *Department of Medicine, New York University College of Medicine* Previous observations (Ralli, *Endocrinology*, 1946) have shown that following adrenalectomy in rats survival was greatly prolonged when large amounts of pantothenic acid were added to the diet (4 to 6 mg daily) The present studies are concerned with establishing the minimal dose of pantothenic acid required for prolonged survival Observations have also been made on the total white blood cell count and the per cent of lymphocyte and polymorphonuclear cells in normal rats on the diet deficient in pantothenic acid and in rats after adrenalectomy when the diet was either deficient or supplemented with varying amounts

of pantothenic acid. At the present time the doses of pantothenic acid that have been tested are 0.3 mg, 1 mg, and 2 mg daily. On the first two amounts the mean survival was 14 days, with a S D  $\pm 4.3$ , and 13 days with a S D  $\pm 1.9$ , respectively. On 2 mg 50 per cent of the rats have survived more than 20 days. This dose is apparently not as effective as the original massive doses used (4 to 6 mg daily). Further experiments on graded doses of pantothenic acid are in progress. The total white blood cell count increased after adrenalectomy to a significant extent in the rats receiving pantothenic acid. There was also an increase in the white blood cells in the adrenalectomized deficient animals, but this was not statistically significant. The total lymphocytes per cubic millimeter were significantly increased in all groups following adrenalectomy.

**Southern peas as a source of protein for growth.** L. R. RICHARDSON, *Department of Biochemistry and Nutrition, A & M College of Texas, College Station.* Southern peas (table varieties of the cowpea) were compared with English peas, pinto beans, lima beans, egg albumen and casein as a source of protein for growth for rats. The legumes furnished all the protein in a purified diet at a level of 10% protein. The vitamins were supplied as an A-D concentrate and as pure compounds. There were 8 rats in each test and the experimental period was four weeks. Cooking did not improve either the Southern peas or the English peas as the same gains were obtained with the raw peas as with the cooked peas in each case. However, the rats fed English peas gained twice as much as those fed Southern peas. Those fed raw lima beans and raw pinto beans lost 12 to 16 grams while those fed cooked lima beans and cooked pinto beans gained 25 and 10 grams, respectively. The gain on Southern peas was equal to that of the rats fed cooked lima beans. Supplementing the cooked legumes with 0.2% of DL methionine increased the gains 2 to 3 times. The average gains were 76, 72, 52 and 46 grams, respectively, when lima beans, English peas, pinto beans and Southern peas were supplemented with methionine. In tests with Southern peas and pinto beans 0.1% of methionine was inadequate and 0.3% was no better than 0.2%. The gain was 56 grams when the diet contained 10% of egg albumen, 34 grams when it contained 10% casein and 96 grams when it contained 20% casein.

**The riboflavin content of the diet and leg trouble in broad breasted bronze turkeys.** L. R. RICHARDSON, R. M. SHERWOOD (by invitation), H. L. GERMAN (by invitation), *Departments of Biochemistry and Nutrition and Poultry Husbandry, A & M College of Texas, College Station, Texas.* The effect of a border line amount of riboflavin on leg disorders in broad breasted Bronze turkeys was tested with four diets. The constant

components of Diets A, B and C were yellow corn meal, wheat shorts, wheat bran, soybean meal, bone meal, vitamins A and D, manganese sulfate and choline. Crystalline riboflavin was added so that Diet A contained an average total of 1.68 mg of riboflavin per pound. Diet B contained 1.33 mg and Diet C contained 2.5 mg. Diet D was high in animal protein and contained an average of 2.04 mg of riboflavin per pound. A total of 7 out of 31 or 22.6 per cent of the turkeys fed Diet A, and 9 out of 38 or 23.7 per cent fed Diet B developed some form of leg trouble by the end of the 23rd week. The symptoms appeared first when the turkeys were 13 weeks old. There were no leg abnormalities in the 64 turkeys which received Diet C or D. Seven of the affected birds were each given 9 doses of 5.0 mg of riboflavin. One did not recover. One recovered in 2 days and the other five recovered in 3 to 4 weeks. Commercial feeds from 2 farms where there was a severe outbreak of leg trouble contained 1.49 and 1.78 mg of riboflavin per pound. These data together with those obtained in the laboratory indicate that leg trouble in turkeys may be due in part to an inadequate supply of riboflavin.

**The effect of pantothenic acid deficiency on acetylation in rats.** THOMAS R. RIGGS (by invitation) and D. MARK HEGSTED, *Dept. of Nutrition, Harvard School of Public Health and Dept. of Biological Chemistry, Harvard Medical School, Boston.* Lipmann *et al.* have studied *in vitro* acetylations of choline and sulfonilamide, and have concentrated the coenzyme (designated coenzyme A) necessary for the reaction (*J. Biol. Chem.*, 160: 173 (1945), 162: 743 (1946), *Federation Proc.*, 5: 145 (1946)). Since these concentrates were found to contain large amounts of pantothenic acid (*J. Biol. Chem.*, 167: 869 (1947)) the present studies were undertaken to demonstrate the need of this vitamin by rats for normal acetylations *in vivo*. p-Aminobenzoic acid (PAB) was used as the test substance throughout. Normal rats were found to acetylate nearly 70% of the amount excreted in 24 hours of a 1 mg. or 2½ mg. dose of PAB administered intraperitoneally. Animals rendered pantothenic acid deficient acetylated only 50% of a 1 mg. dose and less than 40% of a 2½ mg. dose. Simultaneous injection of 1 mg. of calcium pantothenate to deficient animals immediately returned their acetylation to normal. Investigations also were made on the effect on acetylation of added acetate, 24 hour fasting, size of PAB dose, and of thiamine and riboflavin deficiencies.

**Blood constituents of swine in a pantothenic acid deficient condition.** WALTER C. RUSSELL and ARTHUR E. TEERI (now at the University of New Hampshire, Durham, New Hampshire), *Department of Agricultural Biochemistry, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey.* In an attempt

to characterize the pantothenic acid deficiency in swine, determinations were made of certain nitrogenous, lipid, and inorganic constituents of the blood. In comparison with the positive controls, creatinine and serum sodium of the deficient animals showed a tendency toward lower values and for serum chloride, a lower trend was evident in some animals but not in others. Creatine and serum protein values were higher for the deficient animals. For both groups, blood potassium showed a downward trend with age, the value becoming lower for the deficient animals as the deficiency developed. Following an initial rise for both the control and deficient groups, the plasma levels of total lipids, total cholesterol, cholesterol esters, and free cholesterol of the deficient animals became lower at 80 to 90 days of age and remained so throughout the experiment. Between 105 and 164 days of age, the close of the experiment, the average values for total lipids, total cholesterol, cholesterol esters and free cholesterol were 35, 37, 34, and 57% less, respectively, than those of control animals. The significance of these observations in the metabolism of the domestic pig will be discussed.

**The tryptophane requirement for growth of the rat** W D SALMON *Laby of Animal Nutrition, Alabama Polytechnic Inst., Auburn*. The minimum tryptophane required for a normal rate of growth of the Alabama Agricultural Experiment Station (AES) strain of rats was determined. The basal diet contained tryptophane-free casein hydrolysate equivalent to 12% of casein, corn grits 40%, salts 4%, cystine 0.3%, choline chloride 0.2%, sucrose to 100%, and the known essential vitamins except nicotinic acid. The addition of 0.2% DL-tryptophane made the diet adequate for normal growth, 0.18% was inadequate. When 20 mg nicotinic acid was added per kg of diet, 0.1% DL tryptophane was adequate for normal growth. When 9% of casein was substituted for the casein hydrolysate in the basal diet, 0.06% DL tryptophane or a total tryptophane content of 0.205% was adequate for normal growth in the absence of nicotinic acid. However, 15% of casein in the diet did not furnish sufficient tryptophane for normal growth in the absence of nicotinic acid, although this amount of casein supplied 0.235% tryptophane. Moreover, when nicotinic acid was added to the casein hydrolysate diet, supplementation with 0.1% DL-tryptophane was more effective than the same amount of tryptophane supplied in the form of casein.

**Effects of gelatin, glycine and pyridoxine on tryptophane and nicotinic acid metabolism in humans** HERBERT P SARETT and GRACE A GOLD-SMITH *Nutrition Research Laboratory, Tulane University School of Medicine, New Orleans*. The addition of 20 gm of gelatin per day for 10 days to a wheat diet providing about 40 gm of protein and 6 mg of nicotinic acid daily (J Biol

Chem, 167 293, 1947) has no effect upon the urinary excretion of N'-methylnicotinamide (N'-Me) or of 4-pyridoxic acid. There is a decrease in excretion of tryptophane and indoleacetic acid like compounds related to tryptophane. The simultaneous addition of 200 mg of DL-tryptophane with this gelatin results in an increased excretion of N'-Me and tryptophane with no change in 4 pyridoxic acid. When 5 mg of pyridoxine is also incorporated into this diet, the excretion of N'-Me is decreased and that of 4-pyridoxic acid increased. The addition of 20 gm of glycine per day for 10 days to the original wheat diet does not affect the excretion of N'-Me or 4-pyridoxic acid, but does lead to an increase in tryptophane excretion. The glycine ingestion results in larger increases in urinary nitrogen than does the gelatin. Subjects on a corn diet, in which three-fourths of the un-enriched flour of the wheat diet is replaced by corn meal and grits, show somewhat similar responses when pyridoxine, gelatin, or gelatin and tryptophane are added.

**The double strain phenomenon** Biological basis of the nutritional effect in natural resistance to infection HOWARD A SCHNEIDER *The Rockefeller Institute for Medical Research, New York, 21, N Y*. The resistance of mice to infection with *Salmonella typhimurium*, arranged by either genetic or nutritional means, has been found to be dependent upon the presence of some avirulent cells in the heterogeneous infecting inoculum. This fact has led to the separate use of avirulent and virulent cultures in the infection procedure. By controlling the dose and the time interval between the separate injection of avirulent and virulent cultures, the effect of diet on survivorship has been analyzed and an optimum infection procedure selected at which the dietary effect is maximal. The assay of foodstuffs for nutrients capable of influencing an infection has thus become a practicable matter. Such diet-infection experiments are now performed by injecting the mice intraperitoneally with  $10^3$  viable cells of an avirulent strain of *S. typhimurium* and after 24 hours re-injecting the mice with  $10^3$  viable cells of a homologous virulent strain. The results of fractionation of foodstuffs for resistance and susceptibility factors will be presented.

**Effect of phytate and other food ingredients on the absorption of radioactive iron** LEON M SHARPE (by invitation), ROBERT S HARRIS, WENDELL C PEACOCK (by invitation), and RICHARD C COOKE (by invitation) *Departments of Food Technology and Physics, The Massachusetts Institute of Technology and the Walter E Fernald State School*. Fifteen boys, 12 to 17 years old, living in an institution, were fed breakfasts of the following test meals: I, 200 ml water, II, 200 ml milk, III, 200 ml milk plus 234 mg phytic acid as the sodium

salt, IV, 200 ml milk, plus 234 mg phytic acid as in rolled oats (285 gms), V, 200 ml milk, plus 285 gms rolled oats, 150 ml tomato juice, 75 gms egg omelet and 56 gm white bread. Total iron content of all meals was equalized with V at 8.31 mg using  $\text{FeCl}_3$ . Ascorbic acid (32 mg) added to reduce all iron to ferrous form. I and II contained no phytate.  $\text{Fe}^{55}$  and  $\text{Fe}^{59}$  were used alternately. No food was eaten before or until five hours after the test meals. Radioactive iron absorption was calculated from activity of hemoglobin, plasma volume and hematocrits. Maximum radioactivity of the hemoglobin was reached within 10 days after feeding. Average percentage of ingested iron appearing in the hemoglobin was I, 12.4, II, 7.7, III, 0.8, IV, 4.8 and V 2.5. Sodium phytate markedly affected iron absorption and is five times more reactive than that in oatmeal. Milk and milk plus oatmeal decreased iron absorption by 1/3rd and 2/3rd, respectively. Thus phytate free milk interfered with iron absorption nearly as much as phytate rich oatmeal.

**Physical factors influencing dental caries in the cotton rat.** J. KNOX SMITH, E. PORTS ANDERSON, MARIE ZEPPLIN, C. A. ELVEHJEM and PAUL H. PHILLIPS. *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin.* In the production of dental caries in the cotton rat there were certain results which could not be explained in terms of the dietary constituents. Consequently the effect of the physical state of the ration was more thoroughly investigated. Since milk seemed to offer protection against dental caries even when fed along with solid cariogenic rations, diets were used which could be fed in the solid form or liquid form. Dry whole milk (Klim), dry skim milk plus butter, and a casein sucrose diet were used in the study. When any of the above diets were substituted with water and fed, a very great reduction in carious lesions were noted compared to the carious lesions of the control animals. The particle size of the ration was found to be important. A ration containing 67% carbohydrate ( $\frac{1}{2}$  dextrin,  $\frac{1}{2}$  sucrose) diet was used. The dextrin in the diet was fed as coarse or floured. The floured dextrin ration resulted in over twice the amount of dental caries. Caloric restriction studies have also been made. It has been observed that a considerable reduction of carious lesions can be obtained by restricting the food intake of cotton rats.

**A new dairy food.** BARNETT SURE. *University of Arkansas, Fayetteville.* The dairy food consists largely of various milk solids, supplemented with dried brewers' or cultured yeasts, iron and calcium salts, and enriched with vitamins A and D. It has a protein content of 21.8%, a water content of 54%, a mineral content of 4.3%, and a fat content of about 3.5%. Twenty four albino rats have made excellent growth during an eight week period when this food served as the only sources of proteins, fat,

minerals, vitamins and calories. In other words, it is a complete food. Over 200 pounds of this food have been canned to date and the canned product has kept well over four months, being just as fresh and moist after this period as the day after canning. About 400 test meals have been now completed with children and adults ranging in ages from 2 to 75 years who have found the basic food and the food products prepared from it very palatable. The dairy food has been served as fried patties in sandwiches, as dairy loaves, as dairy balls with Spanish sauce and spaghetti, and as croquettes. The loaves and dairy balls have distinct meaty taste. Because of the low cost of the ingredients, it should be possible to make this dairy food available here and abroad at relatively low prices to the consumer.

**Biological value of proteins in food yeasts.** BARNETT SURE and FRANCES HOUSE (introduced by BARNETT SURE). *University of Arkansas, Fayetteville.* This work was carried out on albino rats with the Mitchell method for nitrogen balance studies. Three dried food yeasts (Anheuser-Busch) were studied: brewers' yeast, K, and cultured yeasts, G and 300. On a 5 per cent protein level, the biological values and true digestibilities were respectively, as follows: yeast K, 66.3 and 91.4, yeast G, 69.8 and 91.3, and yeast 300, 71.2 and 92.2. On a 8 per cent protein level, the biological values and true digestibilities were respectively, as follows: yeast K, 65.6 and 88.4, yeast G, 68.1 and 86.4, and yeast 300, 73.1 and 87.9.

**Evaluation of adequate protein nutrition.** P. SWANSON, W. W. SMITH, (by invitation), M. BRUSH, (by invitation), and H. MERRIAM, (by invitation). *The Nutrition Laboratory of the Iowa Agricultural Experiment Station, Ames.* In an attempt to define adequate nutrition with respect to protein in the albino rat, biochemical and physiological measurements that may serve as indices of protein nutrition have been made on 50 healthy stock rats. These data have been correlated with other information obtained in metabolism experiments. The quantity and quality of the dietary supply of nitrogen needed to support this state of nutrition has been studied. Groups of animals have been maintained on diets containing the single protein, lactalbumin, in concentrations ranging from one to 18 per cent of the diet by weight. The lowest quantity of lactalbumin capable of maintaining the animals in the state of nutrition characteristic of the stock controls has been determined. Studies of the dietary importance of various amino acids are in progress at the present time.

**Studies in the rat of inhibitors of pteroylglutamic acid structurally related to this vitamin.** M. E. SWENDSEID (by invitation), E. L. WITTE (by invitation), G. W. MOERSCH (by invitation), O. D. BIRD and R. A. BROWN. *The Research*

*Laboratories, Parke, Davis and Co., Detroit, Michigan* Several inhibitors of pteroylglutamic acid (PGA), active in bacteria, including pterins and crude PGA analogues, were administered to weanling rats to determine whether they produced symptoms characteristic of PGA deficiency, viz reduction in growth rate and decrease in cellular elements of the blood. Alleviation or prevention of these symptoms by administration of PGA was taken as further evidence that the compounds were inhibitors of PGA for the animal. Data are presented on the following compounds: 2,4-diamino-6,7-diphenylpteridine, 2,4-diamino-6,7-dimethylpteridine, 2,4-diamino-6,7-di(1-amino-phenyl)pteridine, 2-amino-4-hydroxy-6,7-diphenylpteridine, N-[4-[(2,4-diamino-6-pteridyl)-methyl]-amino]-benzoyl]-glutamic acid ("4-amino pteroylglutamic acid"), and 4-desoxypteroylglutamic acid. By means of an assay procedure based on the development of leucopenia and/or anemia within a 14-day period in the weanling rat receiving a purified diet containing succinyl-sulfathiazole supplemented with the test compound, "4-amino PGA" was found to be the most efficient PGA inhibitor, with 2,4-diamino-7-diphenyl pteridine also showing a considerable effect. It was found that the amount of PGA necessary to counteract increasing levels of "4-amino PGA" was not proportional to the amount of inhibitor. Some evidence was obtained, on the basis of leucocyte determinations that a compound showing inhibition of PGA at a given level will show PGA activity at a lower concentration. Under our experimental conditions the PGA analogues produced both an anemia and leucopenia whereas the pterine had a preferential effect on the leucocytes. PGA analogues decreased the apparent PGA content of the liver while pterins had no such effect. The changes in leucocyte and hemoglobin concentrations in 150-180 gram rats receiving a purified diet supplemented with "4-amino PGA" are discussed.

**Influence of pteroylglutamic acid on the synthesis and action of the antipernicious anemia factor** ARNOLD D. WELCH, ROBERT W. HEINLE (by invitation), JACK A. PRITCHARD (by invitation), and HERBERT SALIS (by invitation) *Departments of Pharmacology and Medicine, Western Reserve University School of Medicine* Swine fed purified diets containing succinylsulfathiazole and an antagonist of pteroylglutamic acid (PGA), developed leucopenia and macrocytic anemia with megaloblastic hyperplasia of the bone marrow. Remission followed the administration of either PGA or refined liver extract. Relapses developing after liver-induced remissions were refractory to liver extract, but subsequently responded to PGA. Hematologic remission was induced in one pig by

the intramuscular administration of 4 daily 10 mg doses of PGA. Four weeks later the maintained remission was unaffected by 13 additional doses of PGA, but extract prepared from liver (200 gm) removed aseptically was hematopoietically nearly inactive in a pernicious anemic patient. After a month on the same diet, except that crude casein containing extrinsic factor replaced the purified casein, the pig was sacrificed and a second liver extract was prepared. This invoked, in the same patient, a reticulocytosis (12%) without clinical improvement or increase in erythrocyte count, extract prepared from normal pig liver caused a subsequent reticulocytosis (28%) with striking clinical improvement and a prompt increase in the number of erythrocytes. Thus, PGA can elicit a complete hematopoietic response in a pig on a purified diet free of extrinsic factor. If the synthesis of antipernicious anemia factor is involved in this response, little or no hepatic storage of the factor occurs. Administration of extrinsic factor, without added PGA, results in storage of antipernicious anemia factor, but the amount is much less than in a normal animal. The significance of these and other experiments will be discussed.

**Lipid components of skin of dogs on low fat diet and dogs receiving lard** HILDA F. WIESE (by invitation) and ARILD E. HANSEN *Dept. of Pediatrics, Univ. of Texas Medical School, Galveston* Lipid studies have been made on the skin of 29 dogs. These included 9 newborn puppies, 9 young dogs on a low fat diet and 11 control dogs who received fresh lard in the diet. All the dogs on the fat deficient diet manifested desquamation of the skin and dry coarse hair which are characteristic for dogs receiving this diet. The appearance of the skin of the newborn puppies and the dogs receiving fresh lard as 29% of their caloric intake was that of normal healthy animals. The total fatty acids extracted from the skin of newborn puppies showed the highest iodine numbers in the three groups of dogs, while the fatty acids from the skin of dogs on the fat deficient diet were the least unsaturated of the group. There was no difference between the three groups in the average per cent of cholesterol in the skin, but the average cholesterol ester content of the newborn puppy's skin was only  $\frac{1}{3}$  of the other two groups. In addition, the desquamated portion of skin which can be peeled off readily from fat deficient animals showed a composition markedly different from the skin as a whole. Cholesterol and cholesterol ester percentages in this portion of dry skin are more than seven times those of a section of the whole skin. Also the average iodine number of the total fatty acids in the desquamated tissue falls far below that of any other tissue.

## THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

THIRTY SECOND ANNUAL MEETING

Atlantic City, New Jersey, March 15, 16, 17, 18, 19, 1948

(For possible corrections in any of the following abstracts see the next issue)

On the purification of the rickettsia of tsutsugamushi disease (scrub typhus) CHARLES A. BAILLY, FRED H. DIERS and JAMES E. PROFFITT (introduced by A. F. COCA) *National Naval Medical Center, Naval Medical Research Institute, Bethesda, Maryland* A method for the preparation of a relatively pure suspension of rickettsia from the yolk sacs of embryonated hen's eggs infected with *Rickettsia orientalis* has been devised. The suspension has been used as an antigen in serological studies of scrub typhus immune sera by complement fixation tests and as a vaccine in immunity studies of scrub typhus in the white mouse.

The isolation and properties of specific antigens from variants of *Shigella sonnei* EDGAR E. BAKER (by invitation), ELI PERLMAN and WALTHER F. GOEBEL *The Rockefeller Institute for Medical Research, and the Mt. Sinai Hospital* Variants of *Shigella Sonnei* have been described which are known as Phase I (smooth), Phase II, and Rough, and are serologically and morphologically distinct. Specific antigens have been isolated from the Phase I and II variants which are electrophoretically homogeneous. These antigens appear to be lipocarbohydrate-proteins which are toxic and highly antigenic in experimental animals. The gross chemical properties of the two antigens are similar to those obtained from the Flexner group of dysentery bacilli, yet their immunological properties are distinct and specific. The Phase I antigen contains 5.98 and 2.3 per cent nitrogen and phosphorus respectively. The Phase II antigen contains 5.5 per cent nitrogen and 3.1 per cent phosphorus. Antisera prepared by injecting groups of rabbits with minute amounts of the purified antigens agglutinate specifically the homologous microorganisms and show no serological crossing with organisms of the heterologous phase. From the results of these experiments it appears that this phase variation is associated with a change in the enzyme systems of the microorganism which govern the synthesis of the specific somatic antigen.

A critical analysis of the performance of the photorefractometer in the measurement of serological and other turbid systems. ELLIS BOLTON (by invitation), CHARLES LEONE (by invitation) and ALAN BOYDEN (by invitation) *Bureau of Biological Research and Department of Zoology, Rutgers University* The Libby photorefractometer (photometer) is a simple, effective instrument for the measurement of turbid systems. Since it is used as a quantitative tool it is of im-

portance to determine its limitations for the task of providing the quantitative data by means of which serological problems may be solved. Toward this end four photometers were used to measure, (1) inorganic precipitates, (2) a non specifically precipitated protein suspension, (3) antigen-antibody precipitates. The results of these measurements reveal some differences in the behavior of relatively simple, homogeneous precipitates (such as those of clay suspensions) when compared with complex antigen antibody precipitates. The difference in dielectric properties of the precipitates and the fact that the specific antigen-antibody precipitate system is a dynamic one may account for these differences in behavior of the inorganic vs the specific precipitate system. In the latter, the changes in particle size and in the rate of size increase, occur in a system of increasing total amount of precipitate and influence each other in such a way that the net turbidity readings are proportional to the actual amounts of precipitate formed ("internal compensation"). Thus it has been found that over usual ranges of antibody concentration total turbidity (curve area) is directly proportional to the total mass of precipitate formed, photorefractometric measurements of antigen antibody precipitates are relatively insensitive to moderate changes in particle size, the upper limit of instrument response depends upon the suspension studied as well as the instrument used.

A study on toxins and antigens of *S. dysenteriae* DANIEL A. BOROFF *Camp Detrick, Frederick, Maryland* Two types of toxin have been described by various investigators working with *Shigella dysenteriae*. One is associated with the somatic antigen and is soluble in diethylene glycol, while the other is an endotoxin that is precipitated from autolysates of these organisms by trichloroacetic acid. Rough variants lack the somatic antigen but possess the endotoxin. Our investigation has revealed, that although the endotoxin from smooth variants induces formation of antibodies capable of protecting animals against the endotoxin of the rough variant, the latter is neither antigenic nor serologically active. Chemical analyses of the two endotoxins showed that while the nitrogen and phosphorus content of both were similar, the endotoxin of the rough variant was poorer in carbohydrate. By means of absorption experiments, the intact organisms, a diethylene glycol extract, or the trichloroacetic acid precipitate from an autolysate

of the smooth variant of *S. dysenteriae* were found to induce and react with identical antibodies. Antisera to these substances were completely exhausted by absorption with any one of the above preparations. These observations are not compatible with the theory of the existence of two or more toxins and the view that the somatic antigen and the endotoxin are distinct antigenic and toxic entities. To explain the phenomena observed, the suggestion is offered that there may be only one dominant antigen in *S. dysenteriae*, consisting of a complex composed of a toxic non antigenic protein portion and a specific carbohydrate substance that is responsible for the serological and immunological activity. Presumably, this complex may split into various proportions of protein and carbohydrate, depending upon the method of treatment, to yield products described by various investigators as distinct components of *S. dysenteriae*.

**Characterization of the "enzymic" action of influenza viruses on human red cells.** B. A. BRIDY (introduced by THOMAS FRANCIS, JR.) *Walter & Eliza Hall Institute, Melbourne, Australia*. Recent publications from this laboratory have shown that changes in human RBC including the removal of virus-receptors and the development of "pan agglutinability" can be produced by filtrates of *Vibrio cholerae* and by the viruses of mumps, Newcastle disease, and influenza. Considerable evidence has been presented which indicates that these alterations of the surface character of human RBC are the functions of an enzyme in the bacterial filtrates and the virus particles. This parallelism to a standard "enzymic" action is most clearly seen in that aspect of the virus-cell reaction in which concomitantly the cell-receptor is destroyed and the virus eluted. It will be shown that two capacities of the influenza group of viruses (a) the agglutination of RBC, and (b) the elution of the virus particles from the RBC with concomitant receptor destruction, can be dissociated by appropriate treatment. The elutability of viruses is influenced in general by the same treatments and in the same direction as the activity of the soluble factor derived from the cholera vibrio. Elutability can be destroyed by heating that is without action on the hemagglutinin titer, such heated virus can be liberated from RBC to which it is adsorbed by the cholera factor. The evidence presented is consistent with the hypothesis that a factor closely resembling the cholera factor is an integral part of the surface of these viruses and that it plays an important role in the initial virus cell interaction.

**The effect of certain enzyme inhibitors on the activity and growth of psittacosis virus.** THOMAS E. BURNETT (by invitation) and ORVILLE J. GOLUB *Camp Detrick, Frederick, Maryland*. The large size of psittacosis virus suggests the presence of

intrinsic enzyme systems required for its activity. Specific group inhibitors were utilized to detect the presence of an effect on —SH groups and other enzyme systems. Incubation of psittacosis virus *in vitro* with these inhibitors at 37°C for one hour and subsequent titrations of the washed virus in embryonated eggs showed complete or almost complete inactivation with para chloromercuribenzoate (0.001 M), hydroquinone (0.01 M), proflavine (0.01 M), atabrine (0.01 M), and mercuric chloride (0.01 M). Iodoacetamide (0.025 M) and o-iodosobenzoate (0.001 M) produced partial inactivation of the virus. Mono-iodoacetic acid, 2,4-dinitrophenol, sodium azide, sodium fluoride, potassium cyanide, PABA, and urethane produced no inactivation. Glutathione or cysteine was shown to prevent or reverse the inactivating effect of para chloromercuribenzoate on the virus. Dependence of the virus on certain extrinsic enzyme systems of its host was suggested by reduced yields of psittacosis virus in tissue cultures which had previously been exposed for one hour to o-iodosobenzoate (0.01 M) or hydroquinone (0.01 M). No significant reduction in yield was observed when the remainder of the compounds listed above were tested. These results point to the importance of —SH containing enzyme systems in the activity of psittacosis virus.

**Excretion of antibody in feces and urine and its absorption from the bowel.** WILLIAM BURROWS *Department of Bacteriology and Parasitology, University of Chicago, Chicago, Illinois*. Antibody is shown to be excreted in both urine and feces of guinea pigs and man immunized with cholera or typhoid vaccine, reaching agglutinin titers of 1:100 in undiluted morning urine or 24 hour specimens, and in saline extracts of feces containing 10–25 mg/ml solids before clarification. Following primary inoculation peak titers are independent of peak serum titer, appearing in feces, urine and serum in that order, suggesting that the excreted antibody is not derived from serum antibody. It is shown that serum globulin is normally excreted by these routes, and antibody titer is a function of the relative proportions of normal and immune globulin. Fecal and urinary titers decline rapidly, disappearing in 3 weeks in the guinea pig and in 9 weeks in man, but may be maintained by periodic reinoculation. Both homologous and heterologous serum antibody are excreted in the urine and feces following passive parenteral immunization. When given by intragastric inoculation, immune globulin is absorbed from the gut as demonstrated by the titration of antibody, including diphtheria antitoxin, and, in the case of heterologous serum antibody, by the presence of the foreign globulin as shown by the precipitin test, in the serum of the recipient. Peak titer in the serum of the recipient appears 10–14 days after intragastric inoculation, suggesting that the absorbed antibody is stored for



a time in tissues other than the blood Secretion in mucus and transport by lymphocytes are suggested as possible mechanisms of passage of antibody into the bowel from the tissues

**Diphtheria toxin-antitoxin reaction in human antisera** MELVIN COHN and A M PAPPENHEIMER, Jr *Dept of Bacteriology, New York University College of Medicine* Using quantitative immunochemical methods to study the diphtheria toxin-antitoxin reaction in hyperimmune rabbit, horse and human antisera it has been shown that (1) the human system resembles that of the rabbit rather than that of the horse (2) The quantitative precipitin curve in both human and rabbit systems can be described by the equation of Heidelberger and Kendall (3) There are no significant differences in the quantitative precipitin curves using antisera obtained from persons showing tuberculin type hypersensitivity (pseudoreactions) to diphtheria toxin and using antisera from non sensitive individuals The ability of human antitoxin to give a precipitin reaction with toxin is destroyed by heating at 56°C for 30 minutes but its protective power is only slightly impaired The altered antitoxin present in heated antisera can be quantitatively precipitated on the rabbit toxin antitoxin precipitate On fractionation of human antisera by means of alcohol at low temperature, following the method of Deutsch et al, diphtheria antitoxin was found largely in the  $\gamma$ -globulin fraction

**Complement fixation with soluble antigens of Plasmodium knowlesi and Plasmodium lophurae** DAVIS, BERNARD D (introduced by JULES FREUND) *From the Public Health Research Institute of the City of New York, Inc, N Y C 9* Soluble antigens prepared from *P lophurae* (duck blood) and *P knowlesi* (monkey blood) fixed complement with sera, frequently in high titer, of animals infected or immunized with the homologous parasites The lophurae antigen also cross reacted with anti-knowlesi monkey sera, but the knowlesi antigen did not react with anti-lophurae duck sera The knowlesi antigen was an  $(\text{NH}_4)_2\text{SO}_4$  precipitate from a hemolysate Lophurae parasites, however, yield little antigen to hemolysis or isotonic extraction The lophurae antigen was prepared by 10% NaCl extraction, which not only eliminates the undesirable stromata and red cell nuclei, but also yields a larger amount of antigen, and a less anticomplementary product, than the original suspension of parasites The lophurae antigen appears to be associated with intracellular particulates, in contrast to the more truly soluble knowlesi antigen, it forms an opalescent solution, its activity is sedimented at 16,000 rpm and is destroyed by lyophilization, and it cannot be extracted from parasites treated with 0.1% formalin It fixed complement when tested with a few human malarial sera Much weaker reactions were obtained

from a comparable precipitate from normal monkey hemolysate, and none from a comparable 10% NaCl extract of normal duck red cells

**Complement activity of sera from healthy and sick children** PAUL F DE GARA and HENRY P GOLDBERG (by invitation) *New York Hospital and the Department of Pediatrics, Cornell University Medical College, New York City* Complement was titrated by the "50 per cent Hemolysis Method" in sera from 107 children (age range 3 days to 15 years) who were well at the time of the test and for a preceding four-week period Titers ranging from 0.0040 to 0.0069 ml were found in 83.5 per cent, higher titers in 6.3 per cent, and lower titers in 10.2 per cent The mean titer was 0.0053 ml  $\pm$  0.00115 There was no relationship between complement activity and age, sex or season at which the specimen was obtained Low complement titers were found in 20 per cent of children who were sick at the time of the test (upper respiratory infections, pneumonia, meningitis, rheumatic fever, nephritis and other diseases)

**Attempt to produce protection against mosquitoes by active immunization** I N DUBIN, J D REESE (by invitation) and LOIS A SEAMANS (by invitation) *Div of Pathology and Bacteriology, Univ of Tennessee College of Medicine, and Dept of Health and Safety, Tennessee Valley Authority* It has been suggested that the tolerance which some persons acquire to the bites of blood-sucking insects is the result of a true immunity Trager demonstrated that ticks incited an acquired immunity after feeding on experimental animals As yet, no true anti insect immunity has been proven in regard to adult mosquitoes The present experiment was undertaken to see whether rabbits could be protected against mosquitoes by active immunization Antigens consisted of (1) 1:100 aqueous suspension of ground-up bodies of female *Anopheles quadrimaculatus*, (2) Seltz filtrate of extract of above suspension by repeated freezing and thawing The antigens were mixed with mineral oil and lanolin Normal and test animals were exposed to hungry mosquitoes under standard conditions The mosquitoes fed equally well on both control and test groups, and were not inhibited in their biting Moreover, the group of rabbits inoculated with the crude suspension of mosquitoes became sensitized to mosquito bites in contrast to normal non sensitive rabbits The cutaneous reactions consisted of large indurated red papules at the site of the bites, they began in about 30 minutes and reached their maximum size at about 5 hours Attempts to desensitize the animals with the two antigens were unsuccessful Passive transfer of the sensitivity to normal animals was also tried, but without success Thus, the course of vaccination afforded no protection to the animals against mosquitoes, but instead produced hyper-

sensitivity to mosquito bites in previously non-sensitive animals

**Studies in hypersensitivity in simian malaria**  
 I N DUBIN *Div of Pathology and Bacteriology, Univ of Tennessee College of Medicine* Antigens prepared from *Plasmodium knowlesi* and *P. gallinaceum* were inoculated intracutaneously into monkeys in the acute and chronic stages of infection with *P. knowlesi* or *P. cynomolgi*. The antigens prepared from *P. knowlesi* consisted of (1) aqueous suspensions of dried parasites (2) aqueous extract of dried parasites at room temperature for 1 hour (complement-fixation antigen used by Dulaney and Stratman-Thomas) (3) aqueous extract of dried parasites in refrigerator for 24 hours (4) aqueous extract of dried parasites by repeated freezing and thawing (5) solution of wet parasite mass in N/10 NaOH and restoration of pH to 8.0 by  $H_2PO_4$ . The antigens prepared from *P. gallinaceum* consisted of (1) aqueous suspensions of "Malarial Antigen (Dried)" Lederle (2) aqueous extract of above dried antigen at room temperature for 1 hour. As controls, preparations were made from hemolyzed blood of normal monkeys and chickens. The results of the tests were negative, no significant cutaneous reactions were found. An attempt was made to sensitize monkeys to malarial antigen by the use of adjuvants such as a mixture of lanolin, mineral oil, dead tubercle bacilli and dried malarial antigen (*P. knowlesi*), again no cutaneous hypersensitivity was demonstrated. Macrophage sensitivity was sought for in tissue cultures by suspending macrophages from normal monkeys and monkeys chronically infected with *P. knowlesi* in plasma containing dried malarial antigen (*P. knowlesi*), no evidence of macrophage sensitivity in the diseased monkeys was elicited. As judged by these experiments, there is no significant development of hypersensitivity in simian malaria, either accompanying infection or following artificial sensitization.

**Studies on Donovanian granulomatosis**  
 2 Preparation of culture antigens and immunologic tests  
 ANNA DEAN DULANEY, KODA GUO and HENRY PACKER *Division of Pathology and Bacteriology, and Preventive Medicine, University of Tennessee College of Medicine, Memphis* Four antigens were prepared from *Donovania granulomatosis* grown on Locke embryonic yolk-medium. These consisted of bacterial suspension, filtrate, boiled filtrate, and dialyzed filtrate antigens. Of these, the bacterial suspension and boiled filtrate antigens proved most satisfactory, and were employed in complement fixation tests with sera from 88 individuals. These sera were obtained from 24 patients with granuloma inguinale established by positive smears, 28 patients with early syphilis, 5 patients with lymphogranuloma venereum, 11 patients with miscellaneous genital lesions, and 20 healthy individuals

with no evidence of venereal infection. The highest order of sensitivity and specificity was obtained with the boiled filtrate antigen. Eighty-three per cent of 24 patients with granuloma inguinale gave positive reactions with this antigen. One weak (1+) non-specific reaction was obtained in the syphilis group with this antigen. In the same group of syphilitics, 6 positive tests, mostly weak in character, were obtained with the bacterial suspension antigen, as well as 5 with the control antigen. No positive tests were obtained with either the boiled filtrate or bacterial suspension antigens in the patients with lymphogranuloma, miscellaneous genital lesions, and healthy controls. Skin tests were also performed with these two antigens but the results were inconclusive. The bacterial suspension appeared to be a better skin test reagent than the boiled filtrate antigen.

**Studies on inhibition of the agents of murine and feline pneumonitis by penicillin**  
 MONROE D. EATON *Department of Bacteriology and Immunology, Harvard Medical School* Infections of the yolk sac caused by the agent of mouse pneumonitis were sterilized by penicillin in doses of 100 units given 24 hours after infection. For a similar complete virucidal effect on the cat pneumonitis virus, 5,000 units given three times at 2, 72, and 144 hours after infection were necessary. When treatment was delayed the dose of penicillin required increased with the time after inoculation at which it was administered, but inhibitory and virucidal effects on the mouse pneumonitis virus were demonstrable with 5,000 units given 4 to 5 days after inoculation. In vitro virucidal effects by crystalline penicillin G at a concentration of 2,000 units per cubic centimeter were not demonstrable. Impurities of unknown nature in crude penicillin were virucidal in vitro for the mouse pneumonitis virus.

**Serological studies on infectious mononucleosis and other conditions with human erythrocytes modified by Newcastle disease virus**  
 ALFRED S. EVANS (by invitation) and EDWARD C. CURNEN *Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.* Serological reactions with human erythrocytes modified by adsorption and elution of Newcastle disease virus (NDV) were studied to investigate the possible relationship of this virus to the causative agent of infectious mononucleosis suggested by Burnet and Anderson. Serum from 5 of 23 patients with infectious mononucleosis agglutinated such NDV-treated cells in higher titre than serum from normal persons or from patients with non-infectious diseases. Similarly high titres were also obtained with serum from 13 of 101 patients with infections other than infectious mononucleosis. Serum from rabbits immunized against Newcastle disease virus or against any of three other immunologically unre-

lated viruses agglutinated NDV-treated cells in higher titre than normal rabbit serum. Human serum and rabbit antiserum against viruses other than Newcastle disease virus which agglutinated NDV treated human red cells in high titre did not specifically inhibit the agglutination of chicken red cells by Newcastle disease virus. The capacity of both human and immune rabbit sera to agglutinate NDV-treated human erythrocytes appeared to be unrelated either to the heterophile antibody of infectious mononucleosis or to the hypothetical agent of that disease.

**The long persistence of *Rickettsiae orientalis* in the blood and tissues of infected animals**

JOHN P. FOX (introduced by PETER K. OLITSKY) *From the Laboratories of the International Health Division, Rockefeller Foundation, New York.* Mice and eastern cotton rats, infected with *R. orientalis* (agent of tsutsugamushi disease) and surviving because they were treated with thionine dyes or because they had been infected by the subcutaneous route, were sacrificed at various intervals after infection. Blood and tissues were tested for infectivity. Parallel studies were made of serum-antibody (complement fixing) and of resistance to a certainly lethal challenge inoculum of homologous rickettsiae. The observations on cotton rats were made for 269 days and those on mice for 610 days. In both animal species, the titer of serum antibody soon rose to a significant level and remained constant at that level during the entire period of observation. Mice, challenged at intervals, consistently resisted the challenge infection. In spite of the evident solid immunity, it was possible to infect passage mice with one or more materials (whole blood or suspensions of brain, liver or kidney) taken from animals sacrificed at any time during the period of observation. Although kidney was the most reliable source of infectivity, whole blood was shown to be infectious up to the 102nd day in cotton rats and to the 610th day in mice. Interest attaches to the findings with blood, not only because of the simultaneous demonstration of infectivity and antibody in many single specimens, but also because of their significance to the problem of the rodent reservoir in tsutsugamushi disease.

**Mode of action of thionine dyes in combating experimental rickettsial infections of mice** JOHN P. FOX and OSLER L. PETERSON (introduced by PETER K. OLITSKY) *From the Laboratories of the International Health Division, Rockefeller Foundation, New York.* A previous report (J. Exper. Med. 85: 543, 1947) contains an account of the remarkable activity of methylene blue and toluidine blue in combating experimental scrub typhus in mice. The present report deals with experiments demonstrating a similar activity of these dyes against infections with murine typhus rickettsiae,

and also with observations bearing on the mechanism of this antirickettsial effect. The localization and multiplication of rickettsiae in treated and untreated animals has been studied under varying conditions of dye administration, and the antirickettsial activity of a series of chemically related compounds has been determined. From these studies, it is suggested that the mechanism of antirickettsial activity is independent of the *in vitro* rickettsiacidal activity of the dyes and only partly dependent upon the irregular rickettsiostatic effect observed *in vivo*. It seems possible that the thionine dyes also operate, by virtue of their ability to mediate intracellular oxidation, to spare the cells from the usual harmful effects produced by rickettsiae. The survey of related compounds further indicates that antirickettsial activity requires some degree of structural specificity.

**Studies on the mechanism of action of furacin (5-nitro-2-furaldehyde semicarbazone)** MORRIS N. GREEN (introduced by STUART MUDD) *Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.* The inhibitory action of furacin on the growth of *Escherichia coli* was antagonized by the amino acids, L arginine, L lysine, DL phenylalanine, L glutamic acid, DL isoleucine and vitamins of the B complex, notably thiamin, nicotinamide, pyridoxine and pantothenate. Experiments with the Warburg respirometer showed that the aerobic dissimilation of glucose was inhibited 45% by furacin. In a similar fashion the anaerobic dissimilation of glucose and pyruvate was inhibited 35% and 70% respectively. The glucose dehydrogenase system of *Escherichia coli* was also inhibited by furacin. These results offer some explanation for the antagonisms exerted by nicotinamide and thiamin, since these vitamins are related to the coenzymes involved in the dissimilation of glucose and pyruvate. Lipmann's observations may indicate a similar relation for pantothenate in the dissimilation of pyruvate. Both tryptophanase activity and arginine decarboxylase, enzymes containing pyridoxal phosphate, were inhibited by furacin 70% and 45%, respectively. Since enzymes containing pyridoxal phosphate as coenzymes are inactivated by carbonyl reagents, the possibility is suggested that furacin may be reduced by bacteria with the formation of semicarbazide.

**Studies on *Donovania granulomatis*** I. LOCKE-embryonic yolk medium for cultivation of the organism. KODA GUO, ANNA DEAN DULANEY, HENRY PACKER *Division of Pathology and Bacteriology and Preventive Medicine, University of Tennessee College of Medicine, Memphis.* One of the strains of *Donovania granulomatis* obtained from Dr. Katherine Anderson was successfully cultivated in Locke embryonic yolk-medium. Equal parts of yolk and Locke solution were mixed

and coagulated in slant by steam. A small amount of liquid which gave a neutral reaction collected at the bottom of the slant. Following inoculation of the Locke-yolk medium with *Donovania granulomatis* from the yolk of fertile eggs sufficient Locke's solution was added to almost cover the slant. The heaviest growth of organisms occurred in the lower levels of the Locke's solution near the slanted egg-material. The morphology of these organisms appeared identical to that observed in organisms cultured in parallel series in fertile eggs. Curved bacillary forms with bipolar granules predominated. "Safety pin" forms resulting from bulging of the central portion were also seen. Examination by darkfield illumination revealed a highly illuminated outer thin cell with markedly refractive bipolar granules. True capsules were not observed. The addition of human serum to the Locke's solution produced chain forms made up of 5 to 8 bacilli, as well as bizarre pleomorphic forms. Upon this simple medium *Donovania granulomatis* has been carried for 34 generations without variation in morphology, and such cultures have been used in the preparation of antigens which gave highly specific and sensitive complement fixation reactions.

**Toxicity for paramecia of sera from cancerous and non-cancerous persons** JAMES A. HARRISON and (by invitation) MACHTELD E. SANO, ELIZABETH

FOWLER, ROBERT H. SHELLHAMER and CAROL BOCHER. *From the Department of Biology, Temple University, and the Temple University School of Medicine and Hospital.* The aim of this work was to verify the report of Roskin (Am. Rev. Soviet Med., Dec., 1946) that the presence of cancers in human beings is accompanied by the occurrence of serum substances which are toxic for paramecia. Eighty sera (from 79 persons) in final dilutions of 1-20, 1-30 and 1-40 were tested with *Paramecium aurelia* on the first, second, third and eighth days after blood collection with and without the addition of fresh guinea pig serum in final dilution of 1-20. Toxicity was judged by the number of survivors from 15 paramecia after 24 hours in the presence of each serum dilution. This series contained sera from 28 persons with malignant neoplastic disturbances and from 51 persons, including 19 hospitalized individuals, with no history of cancer. Among the 28 sera from cancerous persons 8 were definitely toxic for paramecia, 6 were possibly toxic and 14 were definitely non-toxic. Among the 52 sera from non-cancerous persons 13 were definitely toxic, 10 were possibly toxic and 29 were definitely non-toxic. The toxic fraction in these sera was destroyed rapidly at a temperature of 55°C and slowly at 4°C. The addition of fresh complement to heated or cold-stored sera did not, with a single exception, restore or enhance toxicity. Sixty duplicate and triplicate runs on 30 of these

sera indicated that the determinations of toxicity were quite reliable. Essentially similar results were obtained when sera from 29 additional cancerous and non-cancerous persons were tested with several other species of ciliates. It appears then that while human serum frequently contains fractions sharply toxic for paramecia the toxicity is not closely related to the occurrence of cancer.

**Effect of environmental temperature upon sulfadiazine therapy, body temperature and oxygen consumption in pneumococcus infection** JUNG, J. McBROOM (by invitation) and S. M. ROSENTHAL. *Division of Physiology, National Institute of Health, Bethesda, Maryland.* The survival from type I pneumococcus infection in mice under sulfadiazine therapy is significantly influenced by environmental temperature. The optimum is in the neighborhood of 26°C to 31°C, while markedly lowered therapeutic responses are observed at 18°C. Lowered responses also appear at 37°C. No rise in body temperature was found in mice during the course of a fatal pneumococcus septicemia. Upon placing infected animals under slight stress, by exposing them to 18°C, it was found that the oxygen consumption does not exceed levels attained by normal mice and under these conditions a progressive fall in body temperature occurs. This indicates that the fall in temperature is due to inadequate heat production rather than excessive heat loss. The evidence suggests that as a result of the infection certain mechanisms which, either directly or indirectly, control the metabolic processes are damaged to the extent that the metabolic capacity of the animal is impaired. The nature and site of this damage remains to be established.

**Immunochemical estimation of albumin and gamma globulin in normal and pathological cerebrospinal fluid** ELVIN A. KABAT, MURRAY GLUSMAN (by invitation) and VESTA KNAUB (by invitation). *Departments of Neurology and Bacteriology, College of Physicians and Surgeons, Columbia University and the Neurological Institute of New York.* Antisera to crystalline human serum albumin and to purified human gamma globulin are prepared in rabbits and absorbed with heterologous antigen. The antisera are calibrated by addition of increasing known amounts of antigen to a constant volume of antiserum. After one hour at 37°C and 48 hours in the refrigerator, the precipitates are centrifuged off, washed twice in the cold with saline and analyzed for nitrogen. Total N precipitated is plotted against quantity of antigen added. The albumin and gamma globulin in cerebrospinal fluid is determined by adding an appropriate dilution of cerebrospinal fluid to the albumin and globulin antisera such that the total N precipitated will fall on the calibration curve, measuring the total N in the washed precipitates.

and reading off the corresponding albumin and globulin contents from the curves. Analyses are valid only in the region of antibody excess. In ten healthy individuals cerebrospinal fluid albumin and gamma globulin ranged from 11 to 19 and from 1.7 to 3.8 mg per 100 ml respectively. Fifteen of 16 patients with neurosyphilis showed marked increases in cerebrospinal fluid gamma globulin ranging from 5.6 to 11.6 mg per 100 ml. The lowest values occurred in patients who had responded favorably to therapy. Eight of 14 cases of multiple sclerosis also showed significant increases in gamma globulin ranging from 5.6 to 13 mg per 100 ml.

The inactivation of influenza virus by mercurials and reactivation by sodium thioglycolate and BAL. MORTON KLEIN (by invitation), J. H. BREWER (by invitation), J. E. PEREZ (by invitation) and BEATRICE DAY (by invitation). *Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.* and *Hynson, Westcott and Dunning, Baltimore, Md.* The Lee strain of influenza B virus was inactivated after 5 minutes contact at room temperature with 1:4,000 bichloride of mercury, 1:1,000 metaphen, 1:3,000 phenyl mercuric nitrate, 1:100 mercuriochrome and 1:1,000 merodicein. A 1:2,000 solution of merthiolate did not inactivate the virus. Inactivation was determined by loss of infectivity of the virus for 10-14 gram Swiss mice following intranasal instillation. Merodicein possessed some prophylactic activity when instilled intranasally into mice one hour before the virus. A polyvalent bacteriophage active against *Staphylococcus albus* was also inactivated by 1:10,000 bichloride of mercury. Inactivation of the PR8 strain of influenza A virus and the bacteriophage by the bichloride of mercury could be reversed *in vitro* by a 1:50 aqueous solution of sodium thioglycolate or a 1:300 aqueous solution of BAL. Reactivation of the virus was determined by mouse infectivity tests. Reactivation of the bacteriophage was determined by agar plaque counts.

The reactivation *in vivo* of influenza A virus by BAL. MORTON KLEIN (by invitation) and ENRIQUE J. PEREZ (by invitation) (introduced by STUART MUDD). *Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.* Influenza A virus inactivated by bichloride of mercury can be reactivated both *in vitro* and *in vivo* with BAL (2,3-dimercaptopropanol). In the present work we have utilized the *in vivo* reactivation technique to determine whether the adsorption of influenza virus onto the host cells will remove it from the direct action of a chemical agent. A 1:15,000 dilution of bichloride of mercury will completely inactivate influenza virus, though the ability of the virus to become adsorbed onto cells, as determined by chick red

cell agglutination, is but slightly affected. If 0.1 ml of a 1:200 aqueous dilution of BAL is given intramuscularly to 10-14 gram Swiss mice 5-15 minutes before the intranasal instillation of bichloride of mercury inactivated virus, the BAL can reactivate the virus and the mice will die of infection with influenza virus. However, when the inactivated virus is given intranasally, followed in 30 minutes by the intramuscular injection of BAL, no reactivation occurs. Pneumococci, on the other hand, inactivated with bichloride of mercury and injected intraperitoneally into mice, can be reactivated when the BAL is given intramuscularly either before or after the inactivated bacteria. The results suggest that though a chemical agent is active *in vivo* and has a direct action on the virus, the adsorption of the virus onto the host cells will protect it from the action of the compound.

Effect of salt concentration on the agglutination of red cells by influenza virus and antibody. FRANCIS C. LOWELL, MD and MARGUERITE BUCKINGHAM (by invitation). *Evans Memorial Hospital, 65 E. Newton St., Boston, Mass.* The influence of various salt concentrations in the presence of 5 per cent glucose, on agglutination of red cells by influenza A virus (PR8) was studied. No visible agglutination occurred in concentrations of 0.030M NaCl or less and at a concentration of approximately 0.003M, union of virus and red cells was inhibited. Virus was eluted when combination of virus and red cells was permitted to take place and when the electrolyte was then removed by washing in a salt free solution. Concentrations of salt as high as 0.583M failed to cause noticeable inhibition of agglutination of red cells by virus although such salt concentrations completely inhibited agglutination of red cells by antiserum. For comparative purposes, agglutination of red cells by antibody was likewise studied with respect to the effect of various salt concentrations. The results were similar to those which have been obtained by others and differed from those obtained with influenza virus in that interference with agglutination was much less marked with low and much more marked with high salt concentrations.

The effect of  $Mg^{++}$  on the complementary and coagulative activities of blood serum. \* FRANK MALTANER. *From the Division of Laboratories and Research, New York State Department of Health, Albany.* J. O. DE ALMEIDA (by invitation). *From The School of Medicine, University of Sao Paulo, Sao Paulo, Brazil.* Data presented previously suggest that the enhancing effect of  $Mg^{++}$  on the hemolytic and clotting activities of serum is dependent on and secondary to that of  $Ca^{++}$ . In studies of  $Mg^{++}$  treated complement in the quantitative complement fixation test for syphilis the enhancing action of  $Mg^{++}$  was neutralized by the inactivated

human test serum. The result is an increase in the anticomplementary reaction with serum alone essentially proportional to the increase in hemolytic activity of the complement resulting from treatment with  $Mg^{++}$ . Thus, the titer of sera expressed as the ratio of the reaction obtained with serum plus antigen to that with serum alone is not altered by the use of  $Mg^{++}$  treated complement. Failure to take account of the neutralizing effect of test serum for  $Mg^{++}$  treated complement will lead to errors in the evaluation of titer. In parallel tests of the accelerating effect of  $Mg^{++}$  on the clotting activity of complement treated with  $Ca^{++}$  and cephalin, inactivated human sera neutralized also the clotting effect of  $Mg^{++}$ .

**On the kinetics of hemolysis by antibody and complement** MANFRED M. MAYER and CHARLES C. CROFT (by invitation) *Dept. Bacteriology, Johns Hopkins School of Hygiene and Public Health*. Quantitative studies of the kinetics of immune lysis of sheep erythrocytes have yielded evidence indicating that the action of hemolytic antibody resembles that of an enzyme, while complement appears to play the role of a co factor which is used up in the process. It is thought that complement may furnish the energy which enables antibody to perform its hemolytic function.

**Reactivation of overneutralized influenza virus in chicken embryos** A. P. MCKEE (by invitation) and WM. M. HALE *Univ. of Iowa, Dept. of Bacteriology, Iowa City, Iowa*. The addition of concentrated, heat-inactivated homologous virus to influenza virus type A (PR8), that was overneutralized from 50 to 10,000 times demonstrated that the neutralized virus could be reactivated. This same strain of virus could be recovered from either convalescent or artificially actively immunized mice for a longer period of time by the use of the concentrated, inactive virus than without it. Chicken embryos were used in all cases to detect the active virus. The virus used to affect reactivation was concentrated either by adsorption on and elution from chicken red cells or by evaporation after dialysis. Earlier studies concerning the effect of heat ( $57^{\circ}C$ ) on the hemagglutination and antibody binding activities of the virus were extended. As a result of heating, the hemagglutination activity of both type A (PR8) and type B (Lee) influenza virus was progressively decreased when the virus had been concentrated by the red cell method. The hemagglutinating activity of type B (Lee) was not materially affected, however, if it was concentrated by evaporation. The antibody binding activity of type A (PR8) virus but not type B (Lee) virus was diminished by the same heat treatment regardless of the method used to concentrate the virus.

**Adaptation of poliomyelitis strains to rodents with a word on nomenclature** JOSEPH L. MELNICK

and ROBERT WARD (by invitation) *Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.* This report is concerned with the adaptation of two additional strains of poliomyelitis virus to rodents: the Yale SK strain isolated in 1938 from the stools of a non-paralytic poliomyelitis patient in New Haven, and the Ph strain, isolated in 1913 from a fatal case in the Middle East. The latter strain was established in rodents with material derived from the spinal cord as well as material derived from the colon contents of this patient. Primary transfers from monkeys were made directly to mice and to cotton rats. Several serial transfers were necessary to produce the disease regularly in rodents and to standardize the titration end-point. The disease in mice is similar to that produced by Armstrong's Lansing strain and is characterized by flaccid paralysis of one or more limbs. Both strains remain infectious for monkeys. Infected mouse as well as cotton rat CNS has been shown to have a hundred to thousandfold higher titer in monkeys than in rodents. The disease produced in monkeys with the rodent adapted strain is a replica of the disease produced by the original parent strain. After adaptation in rodents, immunological relationships with the parent strains are retained. Both strains cross immunologically with each other, and with the Lansing strain, but are immunologically distinct from two other "non-adaptable" strains, the 1944 North Carolina strain and the 1945 Philippine Islands strain. Although ready means of designating strains immunologically as Poliomyelitis A, B, or C are not yet available, certain aspects concerning nomenclature and classification will be discussed.

**The production of anti-rabbit hemolysin (rabbit-erythrolysin) in sheep, and its value for complement fixation tests** A. PACHCHAIAN *University of Texas, School of Medicine, Galveston, Texas*. Anti-rabbit hemolysin was produced in sheep by injecting the sheep intravenously and subcutaneously with washed rabbit red blood cells. It was noted that the agglutination titer was much higher than the hemolytic titer. This anti-rabbit hemolysin, plus 5% washed rabbit cells, was used in complement fixation tests. The results were in every case identical with the results obtained from the usual hemolytic system, i.e., positive serum gave positive results, and negative serum, negative results. Other work is in progress to increase the hemolytic titer of anti-rabbit hemolysin. If the titer can be raised considerably, this new hemolysin will be practical for complement fixation tests in places—such as small laboratories—where sheep cannot be kept for a continuously fresh supply of red blood cells.

**The zone of activity of antibodies as determined by the use of radioactive tracers** DAVID PERLSS-

MAN and GEOFFREY KEICHLEY (introduced by A F COCK) *Gates and Ciellin Laboratories of Chemistry and the William G Kerckhoff Laboratories of the Biological Science, California Institute of Technology, Pasadena, Calif* We have found that a method of locating the zone of activity *in vivo* of antibodies prepared against animal tissues is to iodinate the antiserum with iodine containing radioactive iodine in tracer concentrations and determine the disposition of the radioactivity We found that antiovalbumin serum can be iodinated without destroying its ability to precipitate specifically with ovalbumin and that antibody thus specifically precipitated contains iodine Anti-rat kidney serum prepared according to the method of Smadel and similarly iodinated, upon inoculation into rats, localized in the kidney, while iodinated antiovalbumin serum or normal serum showed no such localization This demonstrates that the zone of activity of the anti-kidney serum is the kidney

**Parallel use of the "direct" and "indirect" complement-fixation tests** CHRISTINE E RICE *Division of Animal Pathology, Science Service, Dominion Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec, Canada* The wider application of complement-fixation in serologic surveys of avian and mammalian populations has been hindered by the fact that convalescent sera of certain species fail to exhibit complement-fixation with homologous antigen although the presence of antibody can be detected by other methods Non complement-fixing sera of some of these species however display inhibitive properties when mixed in suitable proportions with convalescent sera from another species which fix complement with the same antigen Methods of titrating the degree of inhibitive activity in such sera have been developed which furnish titers in relatively good agreement with those determined by other serologic methods This method has for convenience been termed the "indirect" complement-fixation test to distinguish it from the usual or "direct" complement fixation test The results obtained by using the two methods in parallel in a number of bacterial and viral systems will be considered

**Further studies on the production *in vitro* from bacteria of substances resembling "natural" agglutinins and precipitins** EDWARD C ROSENOW *From the Rare Metals Institute of the California Institute of Technology, Pasadena, California, and the Longview Hospital, Cincinnati, Ohio* Suspensions, extracts and filtrates of varying strength of different species of bacteria in isotonic NaCl solution were subjected to varying degrees of heat and varying concentrations of different oxidizing agents in the acid, neutral and alkaline range of pH The effects of removal of polysaccharides on agglutinin and precipitin production, of filtering,

dialyzing and distilling on boiling and of adsorption with homologous and heterologous bacteria and of adsorbing agents and of eluting of adsorbed antibody were determined by methods usually employed Specific types of streptococci were isolated in studies of various diseases from the end-point of growth in serial dilution cultures of dextrose brain broth and their specificity was maintained in dense suspensions of glycerol two parts and saturated NaCl solution one part Agglutinin and precipitin titers of solutions of artificial antibody against suspensions and extracts of the respective bacteria were determined at 48°C for 18 hours Specific agglutinins and precipitins were produced usually in very high titer in the acid range of pH from pneumococci and streptococci, from staphylococcus aureus, E coli, B mucosus, and B subtilis roughly proportional to the polysaccharide, to the density of suspensions, extracts and filtrates, to the temperature and to the concentration or activity of the oxidizing agent Of the oxidizing agents used, O<sub>2</sub> under 2,000 lbs pressure, hydrogen peroxide, sodium perborate, calcium dioxide, manganese dioxide, potassium chlorate and permanganate, hydrogen peroxide proved best Thermal agglutinins proved filtrable, dialyzable, and distillable, but after conjugation onto globulin they were no longer dialyzable or distillable and specific titer increased

**A poliomyelitis-like agent in hamsters inoculated with Lansing virus** F K SANDERS (introduced by JOSEPH E SMADEL) *Department of Virus and Rickettsial Diseases, Army Medical Department Research and Graduate School, Army Medical Center, Washington 12, D C* In attempts to obtain concentrations of Lansing poliomyelitis virus greater than those in infected mouse brain, intracerebral passages were made in adult (40-60 grams) or in suckling (5-8 grams) hamsters The virus was carried for 24 serial passages in the brains of adult hamsters The titer of the initial virus, 315th mouse passage, was  $10^{-3}$  and the average titer during the 24 passages in adults was  $10^{-4}$  Virus from the 10th and 19th passages in adult hamsters (A line) were transferred to suckling hamsters (S-1 and S 2 lines) In both instances the titer promptly rose to about  $10^{-6}$  and remained at this level during serial passage in infants Neutralization tests revealed that all three agents (A, S-1, S 2) were identical and cross immunity tests with A and S 1 corroborated this The agent multiplies more readily in infant than adult hamsters The exact identity of the hamster virus is uncertain Anti-Lansing monkey sera gave inconsistent results in neutralization tests with the A and S-1 viruses Moreover, the hamster viruses were unaffected by four separate pools of normal human sera all of which neutralized the Lansing mouse strain Both A and S-1 viruses were neutralized slightly by antisera against GD VII mouse encephalomye

litis, the mean neutralization index in five tests was 40 with hamster virus and 290 with GD VII. It appears not impossible that our hamster virus represents a poliomyelitis-like agent of hamsters. However, no such agent was encountered in 18 consecutive intracerebral passages in hamsters of Fort Bragg fever nor was the virus recovered from the feces of normal adult hamsters. The need for criteria for differentiating various rodent strains of poliomyelitis and poliomyelitis-like viruses is evident.

**The relation of tryptophane utilization to the mechanism of resistance to sulfonamides.** M. G. SEVAG and EDWARD STEERS (introduced by M. G. SEVAG) *Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.* Five different pairs of susceptible and corresponding resistant strains of *Staphylococcus aureus* were studied by determining (1) The tryptophane content of the cells, and (2) the amount of tryptophane consumed during growth. The growth of the susceptible organisms is markedly inhibited by sulfathiazole, whether or not the medium contains added tryptophane. In contrast the resistant organisms show no inhibition of growth by sulfathiazole in these media. Added tryptophane, in the absence of sulfathiazole, is utilized by susceptible and resistant cells whether or not glucose is present. Added tryptophane, in the presence of sulfathiazole, is not utilized by the susceptible but is utilized by the resistant cells. Glucose counteracts to some degree this failure of the susceptible cells to utilize added tryptophane in the presence of sulfathiazole. The tryptophane content of those susceptible cells which do grow in the presence of sulfathiazole indicates that the susceptible cells are capable of synthesizing tryptophane. The utilization of the synthesized or added tryptophane for growth of the susceptible cells is blocked by sulfathiazole, but the utilization of the added or synthesized tryptophane in the resistant cells is not blocked by sulfathiazole. In the absence of added tryptophane glucose does not aid in the utilization of synthesized tryptophane by the susceptible cells. The utilization of tryptophane and glucose simultaneously is concomitant with the decrease in the inhibition of growth by sulfathiazole. The principal difference between the susceptible and resistant strains with respect to tryptophane resolves itself into the failure or ability to utilize the added or synthesized tryptophane in the presence of sulfathiazole.

**Studies on pneumococcal enzymes involved in resistance to drugs.** M. G. SEVAG and JOSEPH S. GORS (introduced by M. G. SEVAG) *Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.* Individual

strains of three types of *Pneumococcus* (I, II and III) were made resistant respectively to sulfonamides, acriflavine, itabrine, optochin and propamidine. Susceptible and resistant forms in the mucoid phase were investigated for their dehydrogenase activity (Thunberg technique) in the presence of glucose, hexose-diphosphate, glycerol, lactate and ethyl alcohol. In general, the resistant strains show a definite decrease in dehydrogenase activity in one or more of these substrates. An analysis of the role of riboflavin metabolism in the resistance and action of these drugs revealed the following general findings: 1) With the exception of the sulfonamides, all the drugs inhibit methylene blue reduction by the susceptible organisms in the presence of glucose. 2) The strains resistant to growth inhibition are also resistant to the inhibition of the glucose dehydrogenase activity by the respective drugs. 3) The inhibition of methylene blue reduction by itabrine, acriflavine and propamidine is counteracted by riboflavin but not by nicotinamide and thiamine. However, the inhibition by optochin is not counteracted by riboflavin. 4) Resistant strains showing a marked decrease in dehydrogenase activity show a marked acceleration of this activity in the presence of added riboflavin but not in the presence of nicotinamide and thiamine. These findings indicate a correlation between flavoprotein metabolism and resistance to acriflavine, itabrine and propamidine. Experiments relating sulfonamides and optochin to flavoprotein metabolism are suggestive but as yet insufficient.

**Immunological studies of cholera filtrates.** E. SINGER, S. H. WEL and S. H. HOU (introduced by Dr. COCK) *From the Central Bacteriological Laboratory, National Institute of Health, Nanjing, China.* The authors confirm Burnet's finding of a substance in cholera filtrates (beef broth containing 1% agar) possessing a destructive specific action against the epithelium of the guinea pig ileum. They find it easily heat labile (50°C for 30 min.) susceptible to acid reaction (pH 5.0 to 6.4 for 24 hrs.) and to ageing (7 to 20 days at room temperature, and to 0.3 per cent formaldehyde). It is antigenic, calling forth specifically neutralizing antibodies in injected rabbits and also in human subjects who have been vaccinated with cholera vaccines. It is not present in autolysates of the vibrios, nevertheless, it is a somatic antigen, since the injection of heat killed washed vibrios stimulates neutralizing antibodies. The authors, referring to Burnet's statement that the factor is a mucin-splitting enzyme recall that other demonstrably toxic substances have enzymatic properties. They also point out the circumstance that the specifically destructive somatic antigen of cholera presents the properties of an exotoxin rather than the usual somatic antigens.



# AMERICAN ASSOCIATION OF IMMUNOLOGISTS

**Complement-fixation with P knowlesi antigen for diagnosis of malaria** W D SUTLIF (by invitation) and ANNA DEAN DULANEY and W L DAVIS (by invitation) Kennedy Hospital and Division of Pathology and Bacteriology, University of Tennessee College of Medicine, Memphis The complement-fixation test for malaria, employing P knowlesi antigen, was used during the year 1947-1948 to determine how often confirmation of the diagnosis could be obtained when the blood film was negative. One hundred and twenty one tests were carried out for 103 patients. The tests were positive in 15 of 55 sera from patients without parasitemia but with histories of attacks within one year. Complement fixation tests furnished the sole objective evidence of infection in this group. In patients with parasitemia 24 of 28 gave positive complement fixation reactions. All tests were negative in cases with no history of malaria longer than one year before examination. Doubtful reactions occurred in 13 sera. Two of these were in patients with parasitemia and five in patients with history of recent malaria. Five were associated with other diseases which at time may simulate malaria, especially Brucellosis. It was concluded that positive complement fixation tests were of value in the diagnosis of malaria in patients without demonstrable parasitemia.

**Role of the spleen and phagocytosis in the quinine treatment of malaria** W H TALIAFERRO, L G TALIAFERRO (by invitation) and F E KELSEY University of Chicago, Chicago, Illinois In line with work on other infections, we have found that quinine is less effective against *Plasmodium gallinaceum* in splenectomized than in normal chickens. Experiments involving infection at various intervals after quinine indicate that quinine acts only as long as it can be found in the blood. The drug acts chiefly by inhibiting asexual reproduction of the parasites. Quinine does not increase phagocytosis by the macrophages of the spleen, liver and bone marrow as ascertained from tissue sections and smears taken at frequent intervals during treatment. Therefore, quinine stimulates neither innate nor acquired immunity because both have been shown to be associated with phagocytosis by these macrophages. Splenectomy raises the quinine blood levels following a standard intravenous dose. A study of the parasitemia in splenectomized chickens given quinine indicates that splenectomy reduces acquired, but not innate immunity. It is concluded that three independent factors operate during quinine treatment of malaria, i.e., (1) innate immunity, (2) acquired immunity and (3) the antimalarial activity of quinine. Removal of the spleen decreases acquired immunity to such an extent that any increased antimalarial activity probably resulting from an

increased quinine level following splenectomy is obscured.

**A turbidimetric growth assay method for the determination of the relative bactericidal activities of sera** HENRY P TREFFERS and KATHERINE E YAW (by invitation) Section of Immunochemistry, Dept of Bacteriology and Immunology, Yale University School of Medicine, New Haven The bactericidal action of serum plus complement on microorganisms is generally assayed from plate counts of the number of organisms which survive. The method is wasteful of time and material since generally only a few of the necessary plates give readable data, and the precision leaves much to be desired. In addition, no indication of the course of the experiment can be obtained until the plates have grown out, which does not permit replication before the complement has deteriorated substantially. In the method proposed the number of organisms surviving the action of serum and complement is determined from the amounts of growth obtained on subculture in liquid media. A suitable number of organisms is mixed with immune serum and complement and incubated. At intervals quantitative aliquots are removed and placed into broth. Similar dilutions are made from control tubes containing organisms, serum and inactivated complement. All tubes are incubated until a readable density of growth is obtained. For convenience the tubes may be heated to kill the organisms before the readings are made in the colorimeter. The relative amounts of growth obtained in the subcultures are not a function of the incubation time so long as the organisms remain in the logarithmic growth phase. In experiments with *S. dysenteriae* a linear relationship has been obtained between the relative numbers of organisms surviving and the logarithm of the amount of serum added. At present all comparisons are made against a standard serum run at the same time.

**A serological relationship between the virus of encephalomyocarditis and certain strains of poliomyelitis-like viruses** JOEL WARREN (by invitation) and JOSEPH E SMADEL Department of Virus and Rickettsial Diseases, Army Medical Department Research and Graduate School, Army Medical Center, Washington 12, D C Previous reports have indicated that the virus of encephalomyocarditis recovered from a chimpanzee in Florida produced paralytic disease and induced unique lesions in the brains and hearts of rodents and the rhesus monkey. In addition, this small size virus was shown to be immunologically unrelated to some 20 odd viruses including the SK-New Haven and Lansing strains of poliomyelitis. Finally, either this virus or one closely related to it was found to be the cause of a mild aseptic meningitis col-

**FEDERATION PROGRAM**  
**Thirty-Second Annual Meeting**  
**JOINT SESSION OF THE FEDERATION**

*Tuesday, March 16, 1 30 p m*

BALLROOM, CONVENTION HALL

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**Maurice H Seevers, Presiding**

- 1 **Paul R Cannon**, *Department of Pathology, University of Chicago, Chicago, Illinois*  
Tissue Protein Synthesis
- 2 **Louis S Goodman**, *Department of Pharmacology, University of Utah, School of Medicine, Salt Lake City, Utah*  
Pharmacological and Physiological Aspects of Adrenergic Blockade, with Special Reference to Dibenamine
- 3 **C A Elvehjem**, *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin*  
The Nutritional Significance of the Intestinal Flora
- 4 **Robert F Pitts**, *Department of Physiology, Syracuse University, College of Medicine, Syracuse, New York*  
The Renal Excretion of Acid

**MOTION PICTURES**

*Thursday, March 18, 8 00 p m*

ROOM C, CONVENTION HALL

- 1 **H M Sweeney and Biophysics Branch Personnel**, *Aero Medical Lab, Air Material Command, Wright Field, Dayton, Ohio*  
Principles of protection against the effects of negative "G"
- > 2 **E H Wood, G E Montgomery, Jr (by invitation) and J E Geraci (by invitation)**, *Section on Physiology of the Mayo Clinic and Foundation, Rochester, Minnesota*  
A technique for obtaining multiple arterial blood samples applied to the study of cyanosis in man
- 3 **J R Whittier (introduced by F A Mettler)**, *Department of Neurology, Columbia University, College of Physicians and Surgeons*  
Rhesus hyperkinesia by subthalamic lesion
- 4 **G H Algire and F Y Legallais (by invitation)**, *National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda, Md*  
The transparent chamber technique in the mouse in the study of tumor histophysiology

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**FEDERATION "MIXER"**

*Wednesday, March 17, 9 00 p m*

ARENA, CONVENTION HALL

Refreshments—Dancing

Admission by Registration Badge

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# THE AMERICAN PHYSIOLOGICAL SOCIETY

## FIFTY-SEVENTH ANNUAL MEETING

### PHYSIOLOGY A

Tuesday, March 16, 9 a m

Ballroom, Convention Hall

#### Renal Hemodynamics

- 1 Francois C Reubi (by invitation), Henry A Schroeder, and Arnold H Williams (by invitation), Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis

Are there arterio venous shunts in the kidney?

- 2 J H Cort (by invitation) and Donald H Barron, Laboratory of Physiology, Yale University School of Medicine

Neural control of the renal shunt

- 3 Domingo M Gomez (introduced by H W Smith), New York University College of Medicine

Calculation of effective afferent and efferent renal resistance

- 4 Alfred A Bolomey, Ernest F Breed, Alexander Michie, Katherine Michie and Henry D Lawson (introduced by H W Smith), New York University College of Medicine

Renal fraction in normal subjects and in subjects with essential hypertension

- 5 Woodrow Batten (by invitation), Ben C Ogle (by invitation), Carlos Rapela (by invitation) J Roy Hege, Jr, J Maxwell Little and Harold D Green, Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston Salem

Relationship between arterial pressure and renal blood flow

- 6 P J Talso (by invitation), A P Crosley, Jr (by invitation) and R W Clarke, Medical Department Field Research Laboratory, Fort Knox

Effects of the cold pressor test on glomerular filtration and effective renal plasma flow

- 7 S Baez (by invitation), A Mazur and Ephraim Shorr, Department of Medicine, Cornell University Medical College and New York Hospital

Hepato renal factors in circulatory homeostasis XX Antidiuretic action of liver VDM concentrates

- 8 H L White and Doris Rolf (by invitation) Department of Physiology, Washington University School of Medicine, St Louis

Effects of exercise on renal circulation

- 9 J P Kriss, P H Fletcher and M L Goldman (introduced by H A Schroeder) Department of Internal Medicine, Washington University School of Medicine, St Louis

Influence of unilateral adrenalectomy and sympathectomy on homolateral renal function

- 10 David M French, Pedro A Molano and Walter M Booker, Department of Pharmacology, Howard University School of Medicine, Washington

Renal function related to increased intra abdominal pressure in anesthetized and unanesthetized dogs

- 11 Kendrick Hare, Luise Ungewitter (by invitation), Henry L Barnett (by invitation), and Helen McNamara (by invitation), New York Hospital and the Departments of Pediatrics and Anatomy, Cornell University Medical College

Observations on the structure and function of the glomerulus in premature and full-term infants

- 12 W G Kubicek, R B Harvey and F J Kottke, University of Minnesota

The adrenalin sensitivity of the denervated dog kidney

### PHYSIOLOGY B

Tuesday, March 16, 9 00 a m

Room 20, Convention Hall

#### Spinal Cord

- 1 L D Carlson and A W Martin (with design in electronics by R S Bark), Dept of Physiology and Biophysics, School of Medicine, University of Washington

A method for studying reflex activity under varying conditions

- 2 Jeane Siskel Frey (by invitation) and Robert Gesell, Physiology Laboratory, University of Michigan

A comparative study of the effects of several anticholinesterases on central nervous integration

- 3 A van Harreveld and George A Feigen (by invitation), California Institute of Technology, Pasadena

The effect of nicotine on spinal synaptic conduction

- 4 David P C Lloyd and A K McIntyre (by invitation), *Laboratories of The Rockefeller Institute for Medical Research, New York*  
Potentials of dorsal roots and related phenomena

- 5 A K McIntyre (by invitation) and David P C Lloyd, *Laboratories of The Rockefeller Institute for Medical Research, New York*  
Spinal projection of hind limb efferent fibers

- 6 Chandler McC Brooks, J C Eccles (by invitation) and J L Malcolm (by invitation), *Depts of Physiology, University of Otago, Dunedin, N Z, and the Johns Hopkins University*

Responses of inhibited motoneurons

- 7 J E Markee and Maude Williams (by invitation), *Dept of Anatomy, Duke University School of Medicine and Division of Physiology, Women's College of the University of North Carolina, Greensboro*

The separate contribution of functional units in limb muscles during reflex withdrawal and crossed extension

- 8 Edward H Lambert, Luis O Mederos (by invitation) and Mavis P Kelsey (by invitation), *Sections on Physiology and Medicine, Mayo Clinic and Mayo Foundation*

A study of the ankle jerk in myxedema

- 9 Jerome Y Lettvin (introduced by W S McCulloch), *Dept of Psychiatry, University of Illinois*

The path of suppression in the spinal grey matter

- 10 Irvin M Korr and Martin J Goldstein (by invitation) *Still Memorial Research Trust, Kirksville, Mo*

Dermatomal autonomic activity in relation to segmental motor reflex threshold

- 11 R D Teasdale (by invitation), and G W Stavraky, *Dept of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada*

Response of deafferented neurones of spinal cord to impulses reaching them from higher levels of central nervous system

- 2 W Kaufman (by invitation), H M Chernoff (by invitation) and L H Nahum, *Laboratory of Physiology, Yale University School of Medicine*

A method of determining the spread of excitation in the ventricles of the dog's heart

- 3 L H Nahum, H M Chernoff (by invitation) and W Kaufman (by invitation), *Laboratory of Physiology, Yale University School of Medicine*

The nature of unipolar limb leads in the dog

- 4 H M Chernoff (by invitation), W Kaufman (by invitation) and L H Nahum, *Laboratory of Physiology, Yale University School of Medicine*

Derivation of leads I and III in the dog from analysis of unipolar limb leads

- 5 L F Nims, B Kartin (by invitation), H M Chernoff (by invitation) and L H Nahum, *Laboratory of Physiology, Yale University School of Medicine*

Heart temperature and its relation to the E-wave

- 6 Grace E Wertenberger and Roberta Hafkesbring, *Department of Physiology, Woman's Medical College of Pennsylvania*

The effect of serum hypersensitivity reactions upon the electrocardiogram

- 7 Alfred Leimdorfer, *Department of Psychiatry, University of Illinois*

The effect of intravenous sodium cyanide on the electrocardiogram

- 8 Bruno Kisch, Franz M Groedel (by invitation) and Paul R Borchardt (by invitation), *Biological Laboratory, Fordham University*  
Differences in epicardial and endocardial electrograms of rabbit, dog and calf

- 9 William G Turman (by invitation) and Jane Sands Robb, *Department of Pharmacology, Syracuse University College of Medicine*

Experimental A-V block, method and discussion

- 10 Joseph H Gast and Harold L Dobson (introduced by Allen D Keller), *Department of Biochemistry, Baylor University College of Medicine, Houston*

Comparison of the effect of some steroids on the hearts of rats with choline deficiency

## PHYSIOLOGY C

Tuesday, March 16, 9 00 a m

Room 21, CONVENTION HALL

### Heart Electrophysiology

- 1 Jane Sands Robb, *Department of Pharmacology, Syracuse University College of Medicine*  
Inconsistencies between experimental and theoretical electrocardiography

## PHYSIOLOGY D

Tuesday, March 16, 9 00 a m

Room 22, CONVENTION HALL

### Industrial Physiology

- 1 William V Whitehorn, *Department of Physiology, Ohio State University*

Circulatory responses to exposure to barometric pressure of 30 mm Hg

- 2 C R Spealman, E W Bixby (by invitation), J LaRue Wiley (by invitation), Michael Newton (by invitation) and H C Bazett, Department of Physiology, University of Pennsylvania

Performance in warm environments following hemorrhage, albumin infusion, bed rest and exposure to cold

- 3 Henry Longstreet Taylor, Austin Henschel, Josef Brozek and Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota

The behavior of certain characteristics related to exhausting work during recovery from semi starvation

- 4 John Haldé and Winfrey Wynn (by invitation), Department of Physiology, Emory University, Ga

Industrial efficiency as affected by various kinds of foods consumed during the mid morning and mid afternoon rest periods

- 5 Austin Henschel, Henry Longstreet Taylor and Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota

The effect of caloric level of refueling on the recovery of work capacity following semi starvation

- 6 Carleton B Chapman (by invitation), Austin Henschel, John Minckler and Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota

Effect of exercise on renal plasma flow

- 7 Albert A Pollack and Earl H Wood, Section on Physiology, Mayo Clinic, Mayo Foundation

Venous pressure in the human leg during exercise and in various positions

- 8 A T Miller, Jr., Laboratory of Applied Physiology, University of North Carolina

The influence of oxygen administration on the cardiovascular response to exercise

- 9 Peter V Karpovich and Nathan Millman (by invitation), Department of Physiology, Springfield College, Springfield, Mass

Seismography applied to the study of track running

- 10 W W Tuttle, Marjorie Wilson (by invitation) and Kate Daum (by invitation), Departments of Physiology and Nutrition, State University of Iowa

The effect of low thiamine intake on the reaction time of women

- 11 John E Peters (by invitation) and W Horsley Gantt, Pavlovian Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins Hospital

Effect of graded degrees of muscular tension on human heart rate

## PHYSIOLOGY E

Tuesday, March 16, 9 00 a m

Room 17, Convention Hall

### Respiration

- 1 D Saris (by invitation), E A Reed (by invitation), J C Scott, Department of Physiology, Hahnemann Medical College, Philadelphia

Observations on the thoracic wall respiratory reflex

- 2 M G Larrabee and Robert Hodes, Johnson Foundation, University of Pennsylvania

Cyclic changes in the respiratory center

- 3 Herbert L Borison (by invitation), George Clark and S C Wang, Department of Physiology, College of Physicians and Surgeons, Columbia University

Localization of certain spasmodic respiratory responses in the medulla oblongata of the cat

- 4 P O Chatfield and S Mead (introduced by E M Landis), Department of Physiology, Harvard Medical School

The role of the vagi in the crossed phrenic phenomenon

- 5 Joseph N Spencer (by invitation), William B Draper, Thomas M Parry (by invitation) and Richard W Whitehead, Department of Physiology and Pharmacology, University of Colorado Medical Center

Studies on diffusion respiration V The hemoglobin oxygen pump

- 6 Hermann Rahn and Arthur B Otis, Department of Physiology, University of Rochester School of Medicine and Dentistry

Man's respiratory response during acclimatization to high altitude

- 7 J E Geraci (by invitation), George E Montgomery, Jr (by invitation) and Earl H Wood, Section on Physiology of the Mayo Clinic and Foundation

Studies of arterial oxygen saturation in patients with suspected arterial hypoxemia, with use of a modified oximeter

- 8 Morton Galdston and Seymour A Horwitz (introduced by J Murray Steele), Department of Medicine, New York University College of Medicine and Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital

Exchange of carbon dioxide and oxygen in the nasopharyngeal part of the respiratory dead space

- 9 Ward S Fowler (introduced by Julius H Comroe, Jr), Department of Physiology and Pharmacology, Graduate School of Medicine,

*University of Pennsylvania*

Respiratory dead space

- 10 **F N Craig, Jane Stubbs** (*by invitation*) and **F N Marzulli** (*by invitation*), *Physiology Section, Medical Division, Army Chemical Center, Md*

Analysis of combined effects of exercise and carbon dioxide inhalation on pulmonary ventilation in man

- 11 **Donald H Barron**, *Laboratory of Physiology, Yale University School of Medicine*  
CO<sub>2</sub> content of maternal and fetal sheep blood

**PHYSIOLOGY F**

Tuesday, March 16, 9 00 a m

ROOM 18, CONVENTION HALL

**Reproduction**

- 1 **Arthur A Cox**, (*introduced by R R Overman*), *Department of Physiology, University of Tennessee College of Medicine*

Observations on the oxytocic property of human blood

- 2 **Annette LaBelle** (*by invitation*) and **Richard Tislow**, *Biological Research Laboratories, Schering Corporation, Bloomfield, N J*

Effect of lactogenic hormone on estrogen response of immature rats

- 3 **Hubert R Catchpole**, *Department of Pathology, University of Illinois College of Medicine*  
Cell fractionation and gonadotrophin assays of anterior pituitary glands

- 4 **M X Zarrow** (*by invitation*) and **W T Salter**, *Laboratories of Pharmacology and Toxicology, Yale University School of Medicine*  
Comparison of biological and chemical evaluations of sex hormone balance (Pharmacol )

- 5 **F D Humm** (*by invitation*) and **W T Salter**, *Laboratories of Pharmacology and Toxicology, Yale University School of Medicine*  
Chemical studies of sex steroid balance in human subjects

- 6 **George Clark**, *Department of Anatomy, Chicago Medical School, and Yerkes Laboratories of Primate Biology, Orange Park, Fla*

The lack of effect of estrogen on the sex skin of the infant male chimpanzee

- 7 **José Gongora** (*by invitation*) and **Charles D Kochakian**, *Department of Physiology and Vital Economics, University of Rochester*

The *in vitro* metabolism of testosterone to  $\Delta^4$  androstenedione 3,17 and cis-testosterone by rabbit liver homogenate

- 8 **James H Leatham**, *Bureau of Biological Research, Rutgers University*

High protein diets and testosterone propionate as related to plasma and liver proteins in rats

- 9 **Boris B Rubenstein**, *Department of Metabolism Endocrinology Research, Michael Reese Hospital, Chicago*

Vitamin E diminishes the vasomotor symptoms of menopause

- 10 **S R M Reynolds, L M Hellman** (*by invitation*) and **P Bruns** (*by invitation*), *Carnegie Institution of Washington, Department of Embryology, and Department of Obstetrics, Johns Hopkins University School of Medicine*

Uterine contractions effective in dilating the human cervix, recorded by the multichannel strain gage tokodynamometer

- 11 **David I Machi**, *Division of Pharmacology, Sinai Hospital, Baltimore*

Concerning the nature of menstrual poison

- 12 **Landrum B Shettles and Zacharias Dische** (*by invitation*), *Depts of Obstetrics and Gynecology, Anatomy and Biochemistry, College of Physicians and Surgeons, Columbia University*

The composition of polysaccharides of human cervical mucus

- 13 **W A Selle and Otis B Miller** (*by invitation*), *Depts of Physiology and Dermatology, University of Texas Medical School, Galveston*

Fetal gastric secretion of radioiodine applied precutaneously to pregnant animals

**JOINT SESSION OF THE FEDERATION**

Tuesday, March 16, 1 30 p m

BALLROOM, CONVENTION HALL

Program on page 314

**PHYSIOLOGY BUSINESS MEETING**

Tuesday, March 16, 4 15 p m

ROOM 20, CONVENTION HALL

**PHYSIOLOGY A**

Wednesday, March 17, 9 00 a m

BALLROOM, CONVENTION HALL

**Axone and Synapse**

- 1 **Domingo Ampil** (*by invitation*) and **Harry Grundfest**, *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

Relation between synaptic delays and characteristics of preganglionic volleys initiating responses in cat autonomic ganglia

- 2 **Harry Grundfest and Domingo Ampil** (*by invitation*), *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

Preganglionic B fibers to the inferior mesenteric ganglion of the cat

- 3 **Abraham M Shanes**, *Dept of Physiology and Biophysics, Georgetown University School of Medicine, Washington, Marine Biological Laboratory, Woods Hole, Mass, and Bermuda Biological Station*

Medium concentration in relation to the water content and electrical properties of nerves

- 4 **Gordon M Schoepfle**, *Dept of Physiology, Washington University School of Medicine, St Louis*

Nerve excitation as a function of membrane voltage

- 5 **D W Bronk, F Brink and M G Larrabee**, *Johnson Foundation, University of Pennsylvania*

The sequence of functional changes in a neurone during narcosis and anoxia

- 6 **J M Posternak** (*by invitation*) and **M G Larrabee**, *Johnson Foundation, University of Pennsylvania*

Action of narcotics on synapses compared to action on axons in sympathetic ganglia

- 7 **Robert Hodes**, *Dept of Physical Medicine, Graduate School of Medicine, and the Eldridge Reeves Johnson Foundation, University of Pennsylvania*

Conduction velocity of skeleto motor nerve fibers to partially paralyzed muscles in human poliomyelitis

- 8 **H K Hartline**, *Johnson Research Foundation, University of Pennsylvania*

Retinal action potentials of photoreceptor cells and the discharge of nerve impulses in their axons

- 9 **Francis O Schmitt and Eduardo De Robertis** (*by invitation*), *Biology Department, Massachusetts Institute of Technology*

Electron microscope observations of nerve structure

- 10 **Rita Guttman**, *Brooklyn College*

Resistance characteristics of rectifier element in single nerve fibers

## PHYSIOLOGY B

Wednesday, March 17, 9 00 a m

Room 20, CONVENTION HALL

Joint Session with The American Society for Experimental Pathology

See Pathology Program for titles

## PHYSIOLOGY C

Wednesday, March 17, 9 00 a m

Room 21, CONVENTION HALL

### Temperature Regulation

- 1 **Leon Eisenberg** (*by invitation*) and **H C Bazett**, *Physiology Department, University of Pennsylvania*

Further observations on intravascular temperature in man

- 2 **N Kleitman, A Ramsaroop** (*by invitation*), and **T Engelmann** (*by invitation*), *Dept of Physiology, University of Chicago*

Variations in skin temperatures of the feet and hands and the onset of sleep

- 3 **Michael Newton** (*introduced by H C Bazett*), *Dept of Physiology, Medical School, University of Pennsylvania*

Electrocardiographic changes on tilting

- 4 **E S Fetcher, S I Rapaport** (*by invitation*) and **John F Hall** (*by invitation*), *Aero Medical Laboratory, Wright Field*

The physiological basis for the internal ventilation of clothing

- 5 **S I Rapaport** (*by invitation*), **E S Fetcher** and **John F Hall** (*by invitation*), *The Aero Medical Laboratory, Wright Field*

Physiological protection of the extremities from severe cold

- 6 **L Love** (*by invitation*) and **H C Bazett**, *Dept of Physiology, University of Pennsylvania*

Heat loss and blood flow in the feet

- 7 **Adelbert Ames, III** (*by invitation*), **Richard S Griffith** (*by invitation*), **David A Goldthwait** (*by invitation*), **Martin B Macht** (*by invitation*), and **H S Belding**, *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass*

A study of various methods of rewarming men after exposure to extreme cold

- 8 **E F Adolph**, *Dept of Physiology, University of Rochester*

Lethality of cold immersion in rats

- 9 **Mortimer E Bader, Martin B Macht** and **Elizabeth L Pillion** (*introduced by H S Belding*), *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass*

Peripheral vascular effects produced by localized warming of various skin areas

- 10 **Martin B Macht, Mortimer E Bader** and **Elizabeth L Pillion** (*introduced by H S Belding*), *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass*

The effect of acetyl-beta-methyl-choline on blood flow through the hand at low temperatures

- 11 John R Brobeck, *Laby of Physiology, Yale Univ School of Medicine*

Food intake as a mechanism of temperature regulation in rats

### PHYSIOLOGY D

Wednesday, March 17, 9 00 a m

ROOM 22, CONVENTION HALL

#### Respiration

- 1 R W Whitehead, D L G Beshore (*by invitation*), W B Draper, Joseph N Spencer (*by invitation*) and Thomas M Perry (*by invitation*), *Dept of Physiology and Pharmacology, University of Colorado Medical Center*

Studies on diffusion respiration VI Changes in kidney function in dogs

- 2 Marshall Brucer (*by invitation*) and H G Swann, *Dept of Physiology, University of Texas Medical School*

A method of assaying the efficacy of resuscitating procedures

- 3 H G Swann and Marshall Brucer (*by invitation*), *Dept of Physiology, University of Texas Medical School*

The recovery period after resuscitation

- 4 A Sokalchuk (*by invitation*), D Ellis (*by invitation*), and E M Greisheimer, *Dept of Physiology, Temple University School of Medicine*

Pulmonary function as affected by operative positions

- 5 Nello Pace, Enrique Strajman (*by invitation*), and Elaine Walker (*by invitation*), *Division of Medical Physics, University of California*

Influence of age on carbon monoxide desaturation in man

- 6 A Hemingway, E B Brown, G S Campbell, F Gollan and J O Elam, *Dept of Physiology, University of Minnesota*

Chronic hyperventilation in normal human subjects

- 7 Arthur B Otis, Mitzi Suskind (*by invitation*) and Hermann Rahn, *Dept of Physiology, University of Rochester School of Medicine and Dentistry*

Effect of pressure breathing and posture upon the respiratory gas exchange and heart rate

- 8 Ralph W Stacy (*by invitation*), Jack A Hunter (*by invitation*) and F A Hitchcock, *The Laboratory of Aviation Physiology, Ohio State University*

A mass spectrometer for the rapid, continuous analysis of respiratory gases

- 9 A C Barger (*by invitation*), G S Richardson (*by invitation*), and E M Landis, *Dept of Physiology, Harvard Medical School*

A Geiger-Müller counter system for tracer studies of gas exchanges in man

- 10 E H Wood, J E Geraci (*by invitation*) and D L Groom (*by invitation*), *Section on Physiology of the Mayo Clinic and Foundation*

Photoelectric determination of blood oxygen saturation in man

- 11 George E Montgomery, Jr (*by invitation*), J E Geraci (*by invitation*) and Earl H Wood, *Section on Physiology of the Mayo Clinic and Foundation*

Calibration of the Millikan compensated oximeter as used among white and colored persons

### PHYSIOLOGY E

Wednesday, March 17, 9 00 a m

ROOM 17, CONVENTION HALL

#### Enzymology

- 1 Maurice M Rapport (*by invitation*), Arda Alden Green and Irvine H Page, *Research Division of the Cleveland Clinic Foundation*

Enzymatic inactivation of serum vasoconstrictor

- 2 J Maxwell Little *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston Salem*

Two fractions of specific cholinesterase present in normal mouse brain

- 3 Charles D Kochakian and Constance E Stettner (*by invitation*), *Dept of Physiology and Vital Economics, University of Rochester*

A comparison of the effect of testosterone propionate and "growth hormone" on the body and organ weights and composition and the arginase and phosphatases of the kidney and liver of the castrated male mouse

- 4 R H De Meio (*by invitation*), A E Rakoff (*by invitation*), A Cantarow and K E Paschkis, *Jefferson Medical College, Philadelphia*

Mechanism of inactivation of  $\alpha$  estradiol by rat liver "in vitro"

- 5 A M Freedman (*by invitation*), Alice Willis (*by invitation*) and H E Himwich, *Medical Division, Army Chemical Center, Md*

Correlation between signs of toxicity and cholinesterase level of brain and blood during recovery from di-isopropyl fluoro phosphate (DFP) poisoning

- 6 S Spiegelman and John M Reiner, *Dept of*



*Bacteriology and Immunology, Washington University School of Medicine, St Louis*

Protection by substrate against inhibition of enzymatic adaptation by protein denaturants

- 7 Clary J Fischer (by invitation) and Roy O Greep, *Harvard School of Dental Medicine*

Effect of magnesium on alkaline phosphatase as influenced by pH, enzyme concentration and aging

- 8 Emily A Feld (by invitation), Mortimer A Rothenberg (by invitation) and David Nachmansohn, *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

On the mechanism of the action of di isopropyl fluorophosphate

- 9 John M Reiner and S Spiegelman, *Dept of Bacteriology and Immunology, Washington University School of Medicine, St Louis*

The partial purification and some properties of an adaptation stimulating principle from yeast

- 10 Otto H Muller, *Dept of Physiology, Syracuse University College of Medicine*

A polarographic study of the action of carbonic anhydrase at the dropping mercury electrode

## PHYSIOLOGY F

Wednesday, March 17, 9 00 a m

ROOM 18, CONVENTION HALL

### Hypertension and Kidney

- 1 George E Brown, Jr (by invitation) and Earl H Wood, *Section on Physiology of the Mayo Clinic and Foundation*

Effect of tetra ethyl ammonium chloride on intra-arterial blood pressure in patients with coarctation of the aorta

- 2 Irvine H Page, R D Taylor and J J Reinhard (by invitation), *Research Division of the Cleveland Clinic Foundation*

The influence of tetraethyl ammonium, hepatectomy and selective destruction of the nervous system on vascular reactivity

- 3 E A Ohler (by invitation) and G E Wakerlin, *Dept of Physiology, University of Illinois College of Medicine*

Further studies on the treatment of experimental renal hypertension with pargyline hydrobromide

- 4 Arnold H Williams (by invitation) and Henry A Schroeder, *Dept of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis*

The azygotic arterial pressure gradient as a measure of local peripheral resistance

- 5 Norman S Olsen (by invitation), Henry A Schroeder and Melvin L Goldman (by invitation), *Depts of Biological Chemistry and Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis*

Methods for extraction of pressor substances from hypertensive blood

- 6 C A Schaffenburg (by invitation) and H Selye, *Institut de Medecine et de Chirurgie Experimentales, Universite de Montreal*

Blood electrolyte changes in experimental renal hypertension

- 7 Edwin P Hiatt, *Dept of Physiology, University of North Carolina School of Medicine*

Effects of oral cinchona alkaloids on circulation of dogs with experimental hypertension

- 8 Helene Wastl, *Dept of Anatomy, Hahnemann Medical College, Philadelphia*

Influence of tea from leaves of *ceanothus americanus* on blood pressure of hypertensive rats

- 9 B W Zweifach, S Rosenfeld (by invitation) and Ephraim Shorr, *Dept of Medicine, Cornell University Medical College and the New York Hospital*

Hepato renal factors in circulatory homeostasis XVI Vascular changes in mesentery in renal hypertension in rats

- 10 Eric Ogden, *Dept of Physiology, University of Texas Medical Branch*

Influence of dibenamine on renal function of the dog

- 11 Arthur J Dziemian (introduced by W Fleischmann) *Medical Division, Army Medical Center, Md*

The effects of burns on kidney function

- 12 J T Bradbury, O F Kraushaar (by invitation), W E Brown, (by invitation), *Dept of Obstetrics and Gynecology, University of Iowa*

The effect of morphine on urinary volume in women

## PHYSIOLOGY A

Wednesday, March 17, 1 45 p m

BALLROOM, CONVENTION HALL

### Nerve Metabolism

- 1 J Magnes and A Geiger (introduced by H Grundfest), *Dept of Physiology, Hebrew University, Jerusalem, and Dept of Psychiatry, College of Physicians and Surgeons, Columbia University*

Effect of hydrogen and bicarbonate ions on the metabolic rate of the perfused cat brain

- 2 Seymour S Kety, Francis D W Lukens,

**Rachel B Woodford** (*by invitation*), **Merel H Harmel** (*by invitation*), **F A Freyhan** (*by invitation*) and **Carl F Schmidt**, *Depts of Pharmacology, Medicine and Psychiatry, University of Pennsylvania and the Delaware State Hospital*

The effects of insulin hypoglycemia and coma on human cerebral metabolism and blood flow

- 3 **F D Carlson** (*by invitation*), **F Brink** and **D W Bronk**, *Johnson Foundation, University of Pennsylvania*

A method for direct measurement of rate of oxygen utilization by nerve

- 4 **C M Connelly** (*by invitation*) and **D W Bronk**, *Johnson Foundation, University of Pennsylvania*

Measurements of rapid changes in oxygen consumption by nerve following brief periods of stimulation

- 5 **R G Grenell**, **P W Davies** (*by invitation*), and **D W Bronk**, *Johnson Foundation, University of Pennsylvania*

The effects of brain anemia on cortical oxygen consumption in vivo

- 6 **P W Davies** (*by invitation*), **R G Grenell** and **D W Bronk**, *Johnson Foundation, University of Pennsylvania*

The time course of in vivo oxygen consumption of cerebral cortex following electrical stimulation

- 7 **L L Boyarski**, **S Postel** and **A Rosenblatt** (*introduced by R W Gerard*), *Dept of Physiology, University of Chicago*

Enzyme inhibitors on conduction and respiration of frog nerve

- 8 **V B Brooks** and **R E Ransmeier** (*introduced by R W Gerard*), *Dept of Physiology, University of Chicago*

Enzyme inhibitors on electric activity of frog brain

- 9 **C Haber** and **L Saidel** (*introduced by R W Gerard*), *Dept of Physiology, University of Chicago*

Glutamic acid in neural activity

- 10 **B Libet**, *Dept of Physiology, University of Chicago*

Adenosinetriphosphatase in nerve

## PHYSIOLOGY B

Wednesday, March 17, 1 45 p m

ROOM 20, CONVENTION HALL

## Gastric Secretion

- 1 **T L Patterson**, **J Kaulbersz**, **D J Sandweiss** and **H C Saltzstein** (*by invitation*), *Depts of Physiology and Surgery, Wayne*

*Univ College of Medicine, and Harper Hospital, Detroit*

The effect of oophorectomized dogs' urine extract on gastric secretion

- 2 **C R Robertson** (*by invitation*) and **M I Grossman**, *Dept of Clinical Science, Univ of Illinois College of Medicine*

Potential of the gastric secretory response to histamine by parasympathomimetic drugs

- 3 **A Littman** (*by invitation*) and **M I Grossman**, *Dept of Clinical Science, Univ of Illinois College of Medicine*

Action of antacids in the human stomach, results with zirconium phosphate

- 4 **M I Grossman**, **C R Robertson** (*by invitation*), and **W L Anderson** (*by invitation*) *Dept of Clinical Science, Univ of Illinois College of Medicine*

Inhibition of gastric secretion by "histamine"

- 5 **E Hale** (*by invitation*) and **M I Grossman**, *Dept of Clinical Science, Univ of Illinois College of Medicine*

Studies on the mechanism of "acid rebound" in gastric acidity

- 6 **Franklin Hollander** and **Frances U Lauber** (*by invitation*), *Gastroenterology Research Laboratory, Mt Sinai Hospital, New York City*

The pH of gastric mucous secretion after equilibration in vitro with alveolar air

- 7 **Gladys R Bucher** and **Arthur Anderson** (*by invitation*), *Dept of Biochemistry, Vanderbilt University*

The uropepsin output in cats given histamine caffeine in beeswax

- 8 **S A Komarov**, **Harry Shay** and **Herman Siple** (*by invitation*), *Fels Research Institute, Temple University School of Medicine*

Secretion of mucin in response to sham feeding and histamine stimulation

- 9 **Leslie E Edwards** and **Carolyn Trowbridge Edwards** (*introduced by R W Ramsey*), *Dept of Physiology and Pharmacology, Medical College of Virginia*

A method for the study of gastric secretion in vitro

- 10 **Carolyn Trowbridge Edwards** and **Leslie E Edwards** (*introduced by R W Ramsey*), *Dept of Physiology and Pharmacology, Medical College of Virginia*

Factors influencing in-vitro secretion of pepsin

- 11 **Charles A Winter** and **Charles W Mushett** (*by invitation*), *Merck Institute for Therapeutic Research, Rahway*

Gastrointestinal and hematological responses in dogs to large doses of histamine and an antihistaminic drug

- 12 A H Ryan, *Dept of Physiology and Pharmacology, Chicago Medical School*  
Sympathetic reaction in peptic ulcer

- 13 J Katz (*by invitation*), Robert L Dryer (*by invitation*), W D Paul (*by invitation*) and J I Routh, *Depts of Biochemistry and Internal Medicine, College of Medicine, Univ of Iowa*

Isolation and assay of an anti secretory substance from duodenal mucosa (Biochem)

### PHYSIOLOGY C

Wednesday, March 17, 1 45 p m

ROOM 21, CONVENTION HALL

#### Temperature Regulation

- 1 V E Hall, R Grant (*by invitation*) and J Field, *Dept of Physiology, Stanford University*

The influence of substances affecting body temperature on thermal polypnea

- 2 J Field, C N Peiss (*by invitation*), and V E Hall, *Dept of Physiology, Stanford University*

The influence of substances affecting body temperature on oxygen consumption and glycolysis in brain

- 3 Walter C Randall, Douglas E Smith and Alrick B Hertzman, *Dept of Physiology, St Louis University School of Medicine*

Some cutaneous responses to "reflex heating"

- 4 Douglas E Smith, Walter C Randall and Alrick B Hertzman, *Dept of Physiology, St Louis University School of Medicine*

Some cutaneous responses to "reflex cooling"

- 5 Walter B Shelley, Peter N Horvath (*by invitation*), and Steven M Horvath, *Depts of Dermatology and Physical Medicine, Univ of Pennsylvania School of Medicine*

Inhibition of sweating by means of iontophoresis

- 6 Steven M Horvath, *Dept of Physical Medicine, Graduate School of Medicine, University of Pennsylvania*

Orthostatic hypotension following hot or cold baths

- 7 J F Herrick and J L Bollman, *Div of Experimental Medicine, Mayo Foundation*

The effect of hyperthermia on the flow of thoracic duct lymph

- 8 James L A Roth and John A Frantz (*introduced by J W Heim*), *Physiology Branch, Aero Medical Laboratory, Wright Field*

Metabolic balances in the cold environment  
I Nitrogen and water exchanges

- 9 John A Frantz and James L A Roth (*introduced by J W Heim*), *Physiology Branch, Aero Medical Laboratory, Wright Field*

Metabolic balances in the cold environment  
II Energy exchanges

- 10 S Rodbard and F Sampson (*by invitation*), *Cardiovascular Dept, Research Institute, Michael Reese Hospital, Chicago*

Further evidence for a temperature sensitive mechanism in a poikilotherm (the turtle)

- 11 Harold D Green and Ben C Ogle (*by invitation*), *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College*

Vasodilation produced by etamon, prisol, body warming and spinal anesthesia in normal extremities

### PHYSIOLOGY D

Wednesday, March 17, 1 45 p m

ROOM 22, CONVENTION HALL

#### Shock

- 1 Samuel A Corson and Elizabeth O'Leary, with the technical assistance of Opal Cain, *Depts of Physiology, School of Medicine, Univ of Minnesota and Howard University*

The influence of sodium salts on the extracellular space in experimental hypoproteine-mic edema

- 2 R R Overman, C P Tharp (*by invitation*) and A H Tuttle (*by invitation*), *Dept of Physiology, Univ of Tennessee College of Medicine*

Alterations in fluid and ionic distribution in various patho-physiological conditions

- 3 C P Tharp (*introduced by R R Overman*), *Dept of Physiology, University of Tennessee College of Medicine*

Comparison of mannitol and thiocyanate volumes in several patho physiological conditions

- 4 N R Joseph (*by invitation*), C I Reed and I E Stock (*by invitation*), *Dept of Physiology, College of Medicine, and Dept of Chemistry, College of Pharmacy, University of Illinois*

Electrochemical determination of ionic diffusion through the synovial membrane

- 5 E W Bixby (*introduced by H C Bazett*), *Physiology Dept, Medical School, University of Pennsylvania*

The recovery of sodium thiocyanate from whole blood and plasma as related to the measurement of extracellular fluid volumes

- 6 W D Collings, C J Martin (*by invitation*) and G R Walters (*by invitation*), *Dept of Physiology, State University of Iowa*

A method for constant, long-term intravenous infusion of the unanesthetized dog

- 7 **John S Kirk** (introduced by K E Jochim), *Dept of Physiology, Univ of Kansas*

Visceral capillary permeability changes produced in the dog by anaphylactic shock and venous stasis

- 8 **Parke H Woodard** (introduced by K E Jochim), *Dept of Physiology, Univ of Kansas*  
Anaphylactic shock in a restricted circulation system excluding the liver in the dog

- 9 **Louis Moreau** (by invitation), **Marvin Bahstocky** (by invitation), and **L V Heilbrunn**, *Zoological Laboratory, University of Pennsylvania*

Studies of shock in frogs I Shock due to electrical injury

- 10 **Monica Reynolds** (introduced by Magnus I Gregersen), *Dept of Physiology, College of Physicians and Surgeons, Columbia University*

An analysis of the effects of large volumes of isotonic saline on the circulation of severely hemorrhaged dogs

- 11 **R C Ingraham** and **Franklin Roemhild** (by invitation), and **H Goldberg** (by invitation) *Dept of Physiology, Univ of Illinois College of Medicine*

Further observations on the effect of pentobarbital and of an adrenolytic agent upon the survival of animals subjected to a procedure resulting in experimental hemorrhagic shock

- 12 **J Garrott Allen** and **Willadene Egner** (by invitation), *Dept of Surgery, University of Chicago*

The reaction caused by the intravenous injection of salmine sulfate in dogs

## PHYSIOLOGY E

Wednesday, March 17, 1 45 p m

ROOM 17, CONVENTION HALL

### Carbohydrate Metabolism

- 1 **J Clifford Stickney**, **David W Northup** and **Edward J Van Liere**, *Dept of Physiology, West Virginia University School of Medicine*

Effect of anoxia on the glucose tolerance curve of dogs

- 2 **Edward J Van Liere**, **J Clifford Stickney** and **David W Northup**, *Dept of Physiology, West Virginia University School of Medicine*

Effect of acclimatization on blood sugar response to anoxia

- 3 **M Wierzechowski** (introduced by William H Chambers), *Physiological Institute of the*

*Medical Faculty, University of Lodz, Poland*

The effect of body temperature on the respiratory transformation of intravenous carbohydrates in mytilid anesthetized dogs

- 4 **Roger M Reinecke** and **Paul J Hauser** (by invitation), *University of Minnesota*

Arterio-venous blood sugar studies on the kidney of the eviscerated dog

- 5 **C H Beatty** (introduced by Magnus I Gregersen), *Dept of Physiology, College of Physicians and Surgeons, Columbia University*

The femoral A-V glucose differences following glucose infusion in unanesthetized normal and adrenalectomized dogs

- 6 **R C de Bodo**, **I H Slater** (by invitation), **H F Weisberg** (by invitation), **K F Prescott** (by invitation), *Dept of Pharmacology, New York University College of Medicine*

Adrenaline hyperglycemia in hypophysectomized dogs

- 7 **M Perlmutter** (introduced by G W Thorn) and **R O Greep**, *Dept of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, and the Harvard School of Dental Medicine*

Effect of insulin upon the in vitro glucose utilization and glycogenesis of the diaphragm of normal and pituitarectomized rats Adaptation of this technique as an assay for serum content of insulin and anti-insulin substances

- 8 **Knud Lunbaek** and **James Stevenson** (introduced by C N H Long) *Dept of Physiological Chemistry and Lab of Physiology, Yale University School of Medicine*

The effect of previous carbohydrate deprivation on the carbohydrate metabolism of isolated muscle

- 9 **Lawrence Greenman** and **John C Rathbun** (introduced by T S Danowski), *Dept of Research Medicine, University of Pittsburgh School of Medicine, the Renziehausen Foundation and the Children's Hospital of Pittsburgh*

Studies of galactose and glucose metabolism

- 10 **J D Meyers** (introduced by E A Stead, Jr), *Dept of Medicine, Duke University School of Medicine*

The effects of the intravenous administration of glucose and amino acids on the hepatic blood flow and splanchnic oxygen consumption of man

- 11 **T S Danowski**, *Dept of Research Medicine and the Renziehausen Foundation, University of Pittsburgh School of Medicine*

Alterations in the rate of glycolysis in human blood following the addition of salts

## PHYSIOLOGY F

Wednesday, March 17, 1 45 p m

ROOM 18, CONVENTION HALL

## Endocrinology

- 1 R de Grandpre (by invitation), J L Prado (by invitation), P Dontigny (by invitation), J Leduc (by invitation) and H. Selye, *Institut de Medecine et de Chirurgie Experimentales, Universite de Montreal, Canada*  
Influence of protein hydrolysates on the production of nephrosclerosis and hypertension by anterior-pituitary preparations
- 2 Henry D Lauson, Howard A Eder (by invitation), Francis P Chinard (by invitation), George C Cotzias (by invitation) and Roger L Greif (by invitation), *Hospital of the Rockefeller Institute for Medical Research*  
Estimation of the rate of antidiuretic hormone secretion in normal man
- 3 William O Maddock, Edwin S Jungck (by invitation), and Carl G Heller, *Dept of Physiology, University of Oregon Medical School*  
Antigonadotrophin formation to sheep FSH effectiveness against endogenous gonadotrophic hormones in men
- 4 Savino A D'Angelo, Albert S Gordon and Harry A Charipper, *Dept of Biology, Washington Square College of Arts and Sciences, New York University*  
The effect of inanition on pituitary adrenal function in the guinea pig
- 5 David Rapport, Attilio Canzanelli and Ruth Guild (by invitation), *Dept of Physiology, Tufts College Medical School*  
Effects of the ablation of the pituitary, adrenals and thyroid on liver regeneration, nucleic acid partition, etc
- 6 I Arthur Mirsky, John Wachman (by invitation), C J Podore (by invitation) and R H Broh-Kahn (by invitation), *May Institute for Medical Research of the Jewish Hospital, Cincinnati*  
Insulin excretion in normal man
- 7 R H Broh-Kahn (by invitation) and I Arthur Mirsky, *May Institute for Medical Research of the Jewish Hospital, Cincinnati*  
Insulin inactivation by tissue extracts
- 8 I H Slater (by invitation), R C de Bodo, H F Weisberg (by invitation) and S P Kiang (by invitation), *Dept of Pharmacology, New York University College of Medicine*  
A study of insulin hypersensitivity in hypophysectomized dogs
- 9 William T Salter, MacAllister W Johnston

(by invitation) and Janet Beach (by invitation), *Laboratories of Pharmacology and Toxicology, Yale Univ School of Medicine*  
Extra-thyroidal iodine

- 10 Warren O Nelson and Helen E Wheeler (by invitation), *Dept of Anatomy, University of Iowa College of Medicine*  
Effect of iodine in hypothyroid rats
- 11 S B Barker and H J Lipner (by invitation), *Dept of Physiology, State University of Iowa*  
Determination of protein-bound iodine in biological material
- 12 Willie W Smith, *Laboratory of Physical Biology, National Institute of Health, Bethesda, Md*  
The influence of thiourea and thiouracil on the response of rats to methyl chloride
- 13 Albert S Gordon and Grace F Katsh (by invitation), *Dept of Biology, Washington Square College of Arts and Sciences, New York University*  
The relation of the endocrine gland system to macrophagic activity

## PHYSIOLOGY EDITORIAL CONFERENCE

Wednesday, March 17, 7 30 p m

ROOM 17, CONVENTION HALL

A C Ivy, Chairman

All members interested in the publication affairs of the Society are cordially invited to attend

## Federation "Mixer"

Wednesday, March 17, 9 00 p m

ARENA, CONVENTION HALL

## PHYSIOLOGY A

Thursday, March 18, 9 00 a m

BALLROOM, CONVENTION HALL

## Hypertension

- 1 Isabelle Dougherty and Sister M A C Day (introduced by A B Hertzman), *Dept of Physiology, St Louis University School of Medicine*  
Observations on the peripheral circulation in neurogenic hypertension
- 2 W G Moss (by invitation) and G E Wakerlin, *Dept of Physiology, University of Illinois College of Medicine*  
The role of the nervous system in experimental hypertension in the dog
- 3 G E Wakerlin, John Marshall, Hiroshi Minatoya, Rufus Walker and A Kaplan, *Dept of Physiology, University of Illinois College of Medicine*

Further studies on the treatment and prophylaxis of experimental renal hypertension with renal extracts

- 4 **Robert D Taylor, A C Corcoran and Irvine H Page**, *Research Division of the Cleveland Clinic Foundation*

Effects of denervation on experimental renal hypertension

- 5 **Lena A Lewis and Irvine H Page**, *Research Division of the Cleveland Clinic Foundation*  
Nature of increased serum  $\beta$  globulin content in malignant hypertension and diabetic retinopathy

- 6 **Ronald E Scantlebury** (introduced by T L Patterson), *Dept of Physiology and Pharmacology, University of Arkansas School of Medicine*

Development of chronic hypertension in dogs by the occlusion of the carotid arteries

- 7 **Melvin L Goldman** (by invitation) and **Henry A Schroeder**, *Dept of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis*

The immediate pressor effect of desoxy corticosterone acetate in hypertensive and normotensive subjects

- 8 **Robert E Shipley and O M Helmer**, *Lilly Laboratory for Clinical Research, Indianapolis General Hospital*

Observations on the sustained pressor principle in different animal species

- 9 **O M Helmer, Robert E Shipley and K G Kohlstaedt**, *Lilly Laboratory for Clinical Research, Indianapolis General Hospital*

Further studies on the identity of the sustained pressor principle

- 10 **Henry A Schroeder, Melvin L Goldman** (by invitation) and **Norman S Olsen** (by invitation), *Depts of Internal Medicine and Biological Chemistry and the Oscar Johnson Institute, Washington University School of Medicine, and Barnes Hospital, St Louis*

Pressor substances in hypertensive blood

- 11 **Raymond E Weston, Leon Hellman** (by invitation), **Doris J W Escher** (by invitation) and **Louis Leiter** (by invitation), *Medical Division, Montefiore Hospital, New York*

Effect of low sodium and Kempner diets on renal hemodynamics and electrolyte excretion in hypertensives

- 12 **L Horlick** (by invitation) and **L N Katz**, *Cardiovascular Dept, Research Institute, Michael Reese Hospital, Chicago*

Standardization of a method for the production of experimental atherosclerosis in the chicken

## PHYSIOLOGY B

Thursday, March 18, 9 00 a m

ROOM 20, CONVENTION HALL

### Axone and Synapse Action

- 1 **Otto H Schmitt**, *Depts of Zoology and Physics, University of Minnesota*

Measurement of electrical energy release "impedance" and longitudinal transport in nerve by differential electrode techniques

- 2 **R Beutner and T C Barnes**, *Dept of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia*

A new concept of phase boundary potential applied to the electrophysiology of nerve

- 3 **Birdsey Renshaw and Herbert Rosenbaum** (by invitation), *Dept of Physiology, University of Oregon Medical School*

Does injury to an axon promptly induce altered excitability in its cell of origin?

- 4 **Mortimer A Rothenberg** (introduced by David Nachmansohn), *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

Rate of penetration of electrolytes into nerve fibers

- 5 **Wynne Sharples** (by invitation), **Harry Grundfest** and **David Nachmansohn**, *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

Adenosinetriphosphatase and conduction of the nerve impulse

- 6 **David Nachmansohn and Mortimer A Rothenberg** (by invitation), *Dept of Neurology, College of Physicians and Surgeons, Columbia University*

The difference between pharmacological action and the physiological role of acetylcholine

- 7 **James E P Toman**, *Dept of Physiology, University of Utah School of Medicine*

Some effects of anticholinesterases upon frog sciatic nerve

- 8 **Leonard H Elwell** (by invitation) and **John W Bean**, *Physiology Laboratory, University of Michigan*

Effects of positive intrapulmonic pressure on muscular contraction

- 9 **W E Burge**, *Dept of Physiology, University of Illinois*

Consciousness and unconsciousness during anesthesia in relation to brain potential

## PHYSIOLOGY C

Thursday, March 18, 9 00 a m

ROOM 21, CONVENTION HALL

### Aviation Physiology

- 1 **E L Corey**, *Physiological Laboratory, University of Virginia Medical School*  
Pilot metabolism and respiratory activity during varied flight tasks
- 2 **M Mason Guest**, *Wayne University College of Medicine*  
Diet and gastro intestinal symptoms at altitude
- 3 **S W Britton and V A Pertzoff** (by invitation), *Physiological Laboratory, University of Virginia*  
Circulatory reserves shown by animals under acceleratory exposure
- 4 **Milton Joffe** (by invitation) and **F A Hitchcock**, *Laboratory of Aviation Physiology, Ohio State University*  
Studies on deceleration
- 5 **H Jacobs** (by invitation), **A Karstens** (by invitation), and **J P Henry**, *Aero Medical Laboratory, Air Materiel Command, Wright Field*  
The effect of pressure breathing on circulating blood volume (2) Distension of the venous arteries
- 6 **J L Gamble** (by invitation), **R S Shaw** (by invitation), **O Gauer** (by invitation) and **J P Henry**, *Aero Medical Laboratory, Air Materiel Command, Wright Field*  
Studies of the pathological physiology of negative "G" in animals and man
- 7 **R S Shaw** (by invitation), **J L Gamble, Jr** (by invitation), **P J Maher** (by invitation), **J P Henry** and **O Gauer** (by invitation), *Aero Medical Laboratory, Air Materiel Command, Wright Field*  
On the use of venous pressure in the head as a tolerance index of negative "G" in humans
- 8 **Samuel Gelfan and George D Davis** (by invitation), *Laboratory of Physiology, Yale University School of Medicine*  
Explosive decompression of monkeys at extreme altitudes
- 9 **Madeline Fusco** (by invitation), **Henry Mellette** (by invitation) and **Fred A Hitchcock**, *Laboratory of Aviation Physiology, Ohio State University*  
Determination of the etiology of pathological effects of explosive decompression on the lungs of rats
- 10 **Fred A Hitchcock, Floyd M Beman** (by invitation) and **John A Kempf** (by invitation), *Laboratory of Aviation Physiology, Ohio State University*  
Estimation of subcutaneous pressure in animals explosively decompressed to pressures of 30 mm Hg
- 11 **True W Robinson** (introduced by J W Heim), *Physiology Branch, Aero Medical Laboratory, Wright Field*  
Pretreatment against decompression sickness

## PHYSIOLOGY D

Thursday, March 18, 9 00 a m

ROOM 22, CONVENTION HALL

### General Physiology

- 1 **F Brink and J M Posternak** (by invitation), *Johnson Foundation, University of Pennsylvania*  
Thermodynamic analysis of the relative effectiveness of narcotics
- 2 **Mary Cregar** (introduced by L V Heilbrunn), *Zoological Laboratory, University of Pennsylvania*  
The effect of anesthetics on calcium release
- 3 **A L Hopkins** (introduced by L V Heilbrunn), *Marine Biological Laboratory, Woods Hole*  
Autotomy as a test for toxic factor
- 4 **J Painter and C M Pomerat**, *Tissue Culture Laboratory of the Department of Anatomy and the Psychopathic Hospital, Medical Branch, University of Texas*  
The toxicity of various stimulants of nervous tissue as revealed by tissue culture technique
- 5 **L E Chadwick and V G Dethier** (by invitation), *Medical Division, Army Chemical Corps, Army Chemical Center, Md, and Johns Hopkins University*  
The distribution of response thresholds in studies of insect chemoreception
- 6 **Wilhelm Buschke**, *Ayer Foundation Ophthalmic Research Laboratory Manhattan Eye, Ear and Throat Hospital*  
Epithelial movements in woundhealing in frog corneas
- 7 **Alfred F Bliss**, *Dept of Physiology, Tufts College Medical School*  
A light stable visual purple
- 8 **J H Bodine and L R Fitzgerald** (by invitation), *Zoological Laboratory, State University of Iowa*  
Change in riboflavin during embryonic development
- 9 **J Percy Baumberger, George F Leong** (by invitation) and **Kathleen Bardwell** (by invitation), *Physiology Dept, School of Medicine, Stanford University*  
A further study of the effect of hemoglobin on cellular respiration
- 10 **C C Hassett** (introduced by L E Chadwick), *Medical Division, Army Chemical Corps, Army Chemical Center, Md*  
The utilization of carbohydrates by drosophila melanogaster
- 11 **John O Hutchins, Betty Podolsky** (by invitation) and **T M McMahon** (by invitation),

*Toxicity Laboratory and Dept of Physiology, University of Chicago*

Effects of tris-( $\beta$  chloroethyl) amine on respiration, carbohydrate and protein synthesis, and cell division in *Chilomonas*

- 12 V G Dethier (by invitation) and L E Chadwick, *Johns Hopkins University and the Medical Division, Army Chemical Corps, Army Chemical Center, Md*

The stimulating effect of glycols and their polymers on the tarsal receptors of blow flies

## PHYSIOLOGY E

Thursday, March 18, 9 00 a m

ROOM 17, CONVENTION HALL

### Metabolism

- 1 Philip Dow and Robert H Shuler (by invitation), *Dept of Physiology, University of Georgia School of Medicine*

The basal metabolic rate of Georgia medical students

- 2 Lawrence R Prouty (by invitation), Martha J Barrett (by invitation) and James D Hardy, *New York Hospital and the Dept of Physiology, Cornell University Medical College*

A simple calorimeter for simultaneous determination of heat production and heat loss in laboratory animals

- 3 Grace M Roth and Charles Sheard, *Section on Physiology and Division of Physics and Biophysical Research, Mayo Clinic and Mayo Foundation*

Relation of basal metabolic rate to vasodilatation and vasoconstriction of extremities of normal subjects as measured by skin temperatures

- 4 Jack L Strominger (by invitation) and John R Brobeck, *Laboratory of Physiology, Yale University School of Medicine*

Adjustment of caloric intake of rats to dietary change

- 5 A Lane (by invitation) and A C Ivy, *Dept of Clinical Science, University of Illinois College of Medicine*

Studies on the nutritional effects of heated fats

- 6 M E Hanson (by invitation) and M I Grossman, *Dept of Clinical Science, University of Illinois College of Medicine*

The failure of intravenous glucose to inhibit food intake in dogs

- 7 H C Meng (by invitation) and Smith Freeman, *Dept of Physiology and Dept of Experimental Medicine, School of Medicine, Northwestern University*

Preliminary study of complete parenteral alimentation

- 8 Hazel E Munsell, Robert S Harris and Louis

O Williams (by invitation), with the technical assistance of Louise Guild (by invitation), Gertrude Nightingale (by invitation) and Cynthia Troesch (by invitation), *Nutritional Biochemistry Laboratories, Massachusetts Institute of Technology*

Composition of Central American foods I Honduras

- 9 Leon M Sharpe (by invitation), Robert S Harris, Wendell C Peacock (by invitation) and Richard C Cooke (by invitation), *Dept of Food Technology and Physics, Massachusetts Institute of Technology and the Walter E Fernald State School*

Effect of phytate and other food ingredients on the absorption of radioactive iron

- 10 Nathan W Shock and Marvin J Yiengst (by invitation), *Division of Physiology, National Institute of Health and Baltimore City Hospitals*

Experimental displacement of the acid base equilibrium of the blood in aged males

## PHYSIOLOGY A

Thursday, March 18, 1 45 p m

BALLROOM, CONVENTION HALL

### SYMPOSIUM

#### Physiology of Neuro-Muscular Junctions

JOHN H WELSH, *Chairman*

- 1 John H Welsh, *Harvard University*  
Introduction
- 2 Stephen W Kuffler, *Johns Hopkins University*  
Electrical Aspects
- 3 George H Acheson, *Harvard University*  
Chemical Aspects
- 4 A McGehee Harvey, *Johns Hopkins Univ*  
Clinical Aspects

## PHYSIOLOGY B

Thursday, March 18, 1 45 p m

ROOM 20, CONVENTION HALL

### Cardiac Work

- 1 Doris J W Escher (by invitation), Raymond E. Weston, George Leiner (by invitation), Louis Leiter (by invitation) and Sybil Goldat (by invitation), *Medical Division, Montefiore Hospital, New York*
- The effect of aminophyllin on cardiac output and renal hemodynamics in man
- 2 Joseph R DiPalma and Richard A Reiss (by invitation), *Dept of Physiology, Long Island College of Medicine*
- The mechanics of Starling's law of the heart



- 3 **Richard A Reiss** (*by invitation*) and **Joseph R DiPalma**, *Dept of Physiology, Long Island College of Medicine*  
The effect of changes in cardiac "competence" on right auricular pressure
- 4 **J E Eckenhoff**, **J H Hafkenschiel**, **E L Foltz** and **R L Driver** (*introduced by Carl F Schmidt*), *Dept of Pharmacology and Harrison Dept of Surgical Research, School of Medicine, University of Pennsylvania*  
The influence of hypotension on coronary blood flow, cardiac oxygen metabolism and cardiac work
- 5 **Albert Roos** (*by invitation*), **J O Elam** (*by invitation*), **J F Neville, Jr** (*by invitation*) and **H L White**, *Dept of Physiology and Laboratory of Applied Thoracic Physiology, Washington University School of Medicine*  
Osmetric determination of cardiac output in man
- 6 **W F Hamilton**, **R L Riley** (*by invitation*), **A M Attyah** (*by invitation*), **Andre Courmand**, **D M Fowell** (*by invitation*), **A Himmelstein** (*by invitation*), **R P Noble** (*by invitation*), **J W Remington**, **D W Richards, Jr** (*by invitation*), **N C Wheeler** (*by invitation*) and **A C Witham** (*by invitation*), *Dept of Physiology, University of Georgia School of Medicine, and the Chest Service, Bellevue Hospital, Columbia University*  
Comparison of the Fick and dye injection methods of measuring the cardiac output in man
- 7 **D M Fowell** (*by invitation*), **A P Briggs**, **N C Wheeler** (*by invitation*), **J A Winslow, Jr** (*by invitation*), **J W Remington** and **W F Hamilton**, *Depts of Physiology, Biochemistry and Medicine, University of Georgia School of Medicine*  
Renal and circulatory factors in congestive failure of the circulation
- 8 **John W Remington**, **W F Hamilton**, **Charles R Noback** (*by invitation*) and **Jay J Gold** (*by invitation*), *Dept of Physiology, University of Georgia School of Medicine and Dept of Anatomy, Long Island Medical School*  
Prediction of the stroke volume of man from brachial pressure pulse values
- 9 **Orville Horwitz** (*by invitation*), **Robert L Mayock** (*by invitation*) and **Isaac Starr**, *Dept of Therapeutic Research and the Dept of Medicine, University of Pennsylvania*  
Direct experiments on the relation between the form of the ballistocardiogram and the shape of the systolic velocity curve in the aorta of man
- 10 **J C Handelsman** (*by invitation*), **Richard**

**J Bing** and **L D Vandam** (*by invitation*), *Dept of Surgery, Johns Hopkins University and Hospital*

Physiological adaptations to anoxia in congenital heart disease with cyanosis

- 11 **Gordon C Ring**, **Catharine R Michie** (*by invitation*) and **M J Oppenheimer**, *Dept of Physiology, Temple University School of Medicine*

X ray density changes in the heart recorded by the electrokymograph

- 12 **Edward F Randak** (*by invitation*), **Bert R Boone** (*by invitation*), **George F Ellinger** (*by invitation*) and **M J Oppenheimer**, *Dept of Physiology, Temple University School of Medicine and Heart Disease Control Section, U S Public Health Service*  
Ventricular isometric relaxation phase as measured on the electrokymograph

#### PHYSIOLOGY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM 20, CONVENTION HALL

#### PHYSIOLOGY C

Thursday, March 18, 1 45 p m

ROOM 21, CONVENTION HALL

#### Gastro-Intestinal Functions

- 1 **R F Furchgott** (*by invitation*) and **Ephraim Shorr**, *Dept of Medicine, Cornell University Medical College and The New York Hospital*  
Effect of anoxia on contractility and metabolism of intestinal smooth muscle
- 2 **Emil Bozler**, *Dept of Physiology, Ohio State University*  
The myenteric reflex
- 3 **Daniel A Brody** (*by invitation*) and **J P Quigley**, *Dept of Physiology, University of Tennessee*  
Measurement of gastric tonus in the normal human
- 4 **Kao Hwang** (*introduced by A C Ivy*), *Dept of Clinical Science, University of Illinois College of Medicine*  
Reflex activities and peristaltic function of the esophagus
- 5 **David W Northup**, **J C Stickney** and **E J Van Liere**, *West Virginia University Medical School*  
The effect of carbon dioxide on the motility of the small intestine
- 6 **K L Shapiro** (*by invitation*) and **A C Ivy**, *Dept of Clinical Science, University of Illinois College of Medicine*

- The effect of "carbonation" on the rate of gastric emptying and intestinal absorption of sucrose solution
- 7 **Dean A Collins**, *Dept of Physiology, Temple University School of Medicine*  
Effect of tetraethylammonium on responses of isolated intestine to angiotonin and other substances
- 8 **G M Morton** (by invitation) and **G W Stavraky**, *Depts of Anatomy and Physiology, Faculty of Medicine, University of Western Ontario*  
The effect of intra arterial injection of acetylcholine upon the gastric mucosa of the dog
- 9 **C C Wang** (by invitation) and **M I Grossman**, *Dept of Clinical Science, University of Illinois College of Medicine*  
Effect of secretin and pancreozymin on amylase and alkaline phosphatase of dog's pancreas
- 10 **I J Pincus** (by invitation), **J E Thomas** and **P O Lachman** (by invitation), *Jefferson Medical College*  
The effect of vagotomy on the secretion of pancreatic juice after the ingestion of various foodstuffs
- 11 **Robert E Davis** (by invitation) and **A C Ivy**, *Dept of Clinical Science, University of Illinois College of Medicine*  
Thermal irritation of alimentary mucosa
- 12 **W Barrett** (by invitation) and **B Craver**, *Division of Pharmacology, Research Dept, Ciba Pharmaceutical Products, Inc*  
Effect of ion deficient Locke's solution on guinea pig ileal responses to histamine, acetylcholine, electrical stimulation (Pharmacol)
- 13 **C J Podore** (by invitation), **R H Broh-Kahn** (by invitation) and **I Arthur Mirsky**, *May Institute for Medical Research of the Jewish Hospital, Cincinnati*  
Uropepsin excretion in man
- Effect of diet on the in vitro synthesis of albumin by liver
- 2 **Carl E Anderson** (by invitation), **Robert G Gale** (by invitation) and **C S Robinson**, *Depts of Biochemistry and Medicine, Vanderbilt University*  
The estimation of functional hepatic mass in normal and phosphorus poisoned dogs (Biochem)
- 3 **R W Denton** (by invitation) and **A C Ivy**, *Dept of Clinical Science, University of Illinois College of Medicine*  
Liver regeneration in the rat
- 4 **Edward A Newman** (by invitation) and **Morton I Grossman**, *Dept of Clinical Science, University of Illinois College of Medicine*  
The effect of diet on liver regeneration in partially hepatectomized rats
- 5 **Clara M Szego** and **Sidney Roberts**, *Dept of Physiological Chemistry, Yale University*  
The chemical composition of regenerating rat liver the influence of ovariectomy
- 6 **Ephraim Shorr** and **B W Zweifach**, *Dept of Medicine, Cornell University Medical College and The New York Hospital*  
Hepato renal factors in circulatory homeostasis XIX VLM and VDM mechanisms in nutritional cirrhosis in rats
- 7 **Stephen Leslie** (by invitation), **George H Stueck, Jr** (by invitation), **Harold M Shorr** (by invitation) and **Elaine P Ralli**, *Dept of Medicine, New York University College of Medicine*  
Chemical and physiological changes during water tolerance tests in patients with cirrhosis
- 8 **J F Canepa** (by invitation) and **A C Ivy**, *Dept of Clinical Science, University of Illinois College of Medicine*  
Comparative lipotropic activity of parenterally administered pancreatic extracts in dietary fatty livers
- 9 **A E Groff** (by invitation), **E deF Baldwin** (by invitation) and **S E Bradley**, *Dept of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York*  
Hepatic arterio-venous oxygen differences in patients with normal and diseased livers
- 10 **Philip K Bondy**, **Walter H Sheldon** and **Lillian Evans** (introduced by **James V Wuren**), *Depts of Medicine and Pathology, Emory University School of Medicine*  
Quantitative glycogen determinations on specimens of human livers obtained by needle biopsy

### PHYSIOLOGY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM 20, CONVENTION HALL

### PHYSIOLOGY D

Thursday, March 18, 1 45 p m

ROOM 22, CONVENTION HALL

### Liver Physiology

- 1 **Attilio Canzanelli**, **David Rapport** and **Ruth Guild** (by invitation), *Dept of Physiology, Tufts College Medical School*
- 11 **N R Brewer** (introduced by **A B Luckhardt**) and **R L Gunning**, *Dept of Physiology, University of Chicago*

The secretory pressure of the liver of the chicken

- 12 **W J Snape** (by invitation), **C W Wirts** (by invitation), **L L Miller** and **A Cantarow**, *Jefferson Medical College*

Excretion of bilirubin and bromsulfalein by the liver

### PHYSIOLOGY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM 20, CONVENTION HALL

### PHYSIOLOGY E

Thursday, March 18, 1 45 p m

ROOM 17, CONVENTION HALL

### Brain Physiology

- 1 **E A Spiegel** and **H T Wycis** (by invitation), *Dept of Experimental Neurology, Temple University School of Medicine*

Electroencephalographic studies following thalamic lesions in humans

- 2 **F E Nulsen**, **S P W Black** and **C V Drake** (by invitation), *Laboratory of Physiology, Yale University School of Medicine*

Inhibition and facilitation of motor activity by the anterior cerebellum

- 3 **Ray S Snider** (by invitation) and **H W Magoun**, *Dept of Anatomy, Northwestern University School of Medicine*

Facilitation produced by cerebellar stimulation

- 4 **Joseph Thomas Roberts**, *University of Arkansas School of Medicine and Gallinger Municipal Hospital, Washington*

The role of the vasa nervorum, especially in regard to "referred pain"

- 5 **F H Lewey**, *Peripheral Nerve Study Center, Hospital of the University of Pennsylvania*

Quantitative examination of sensibility in peripheral nerve injuries

- 6 **L W Freeman**, **H B Shumacker, Jr** (by invitation), **E E Wayson** (by invitation) and **N M Stahl** (by invitation), *Dept of Surgery, Yale University School of Medicine*

Conduction of painful impulses from the extremities via the sympathetic nervous system

- 7 **James D Hardy** and **Carl T Javert** (by invitation), *Russell Sage Institute of Pathology, The New York Hospital, and the Depts of Physiology, Gynecology and Obstetrics, Cornell University Medical College*

Experimental investigation of pain intensity in labor

- 8 **Abraham Wiker** and **Karl Frank** (by invitation), *Research Dept, U S Public Health Hospital, Lexington, Ky*

Effects of electroshock convulsions on chronic decorticated cats (Pharmacol)

- 9 **John M Brookhart** and **Russell E Morris** (by invitation), *Institute of Neurology, Northwestern University Medical School*

Antidromic potential recordings from the medullary pyramid of the cat

- 10 **L M N Bach** (introduced by **H S Mayerson**), *Dept of Physiology, Tulane University School of Medicine*

The role of the bulbar facilitatory and inhibitory systems in vasomotor and respiratory activity

- 11 **W Horsley Gantt**, *Pavlovian Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins Hospital*

Effect of satiation on the intensity of the conditional and unconditional salivary secretion

- 12 **J M Essenberg** (introduced by **George Clark**), *Dept of Anatomy, The Chicago Medical School*

The effect of nicotine on maze-learning ability of albino rats

### PHYSIOLOGY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM 20, CONVENTION HALL

### PHYSIOLOGY

#### Motion Pictures

Thursday, March 18, 8 00 p m

ROOM C, CONVENTION HALL

Program on page 2

### PHYSIOLOGY A

Friday, March 19, 9 00 a m

BALLROOM, CONVENTION HALL

### Renal Mechanisms

- 1 **L J Cizek** (by invitation) and **J H Holmes**, *Dept of Physiology, College of Physicians and Surgeons, Columbia University*

Studies of the mechanism of increased chloride excretion during osmotic diuresis

- 2 **Laurence G Wesson**, **W Parker Anslow, Jr** and **Homer W Smith**, *New York University College of Medicine*

The renal excretion of strong electrolytes

- 3 **J C Roemmelt** (by invitation), **O W Sartorius** (by invitation) and **R F Pitts**,

*Dept of Physiology, Syracuse University  
College of Medicine*

Sodium and chloride reabsorption

- 4 R F Pitts, J L Ayer and W A Schiess,  
*Dept of Physiology, Syracuse University  
College of Medicine*

The reabsorption and excretion of bicarbonate in normal man

- 5 Iftakhar Jahan (*by invitation*) and R F Pitts,  
*Dept of Physiology, Syracuse University  
College of Medicine*

The effect of parathormone on the renal tubular reabsorption of inorganic phosphate

- 6 W D Lotspeich (*introduced by R F Pitts*),  
*Dept of Physiology, Syracuse University  
College of Medicine*

Glomerular filtration rate in the adrenalectomized, salt-fed rat

- 7 O W Sartorius (*by invitation*), J C Roemmelt (*by invitation*) and R F Pitts, *Dept of Physiology, Syracuse University College of Medicine*

Changes in renal function in experimental metabolic acidosis in the normal human subject

- 8 W Parker Anslow, Jr, Laurence G Wesson, Jr, Alfred A Bolomey and John G Taylor (*introduced by Homer W Smith*), *New York University College of Medicine*

Chloruretic action of pressor antidiuretic fraction of posterior pituitary extract

- 9 Elizabeth O'Leary (*by invitation*) and Samuel A Corson, with the technical assistance of Opal Cain, Catherine Anderson and Linor Foster, *Dept of Physiology, School of Medicine, University of Minnesota and Howard University*

Renal clearance studies in experimental hypoproteinemic edema

- 10 W Q Wolfson (*by invitation*) and R Levine, *Depts of Biochemistry and Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago*

The transport and excretion of uric acid in man IV The renal mechanism for urate excretion

- 11 Henry L Barnett (*by invitation*), Helen McNamara (*by invitation*), Ruth S Hare (*by invitation*) and Kendrick Hare, *New York Hospital and Dept of Pediatrics, Cornell University Medical College*

Inulin, urea, mannitol and PAH clearance ratios in premature infants

- 12 C Boyd Shaffer, Frances H Critchfield and Charles P Carpenter (*introduced by J M Rogoff*), *Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh*

The renal clearance of some polyethylene glycols in the dog

## PHYSIOLOGY B

Friday, March 19, 9 00 a m

ROOM 20, CONVENTION HALL

### Peripheral Circulation

- 1 Clifford G Gaddy (*by invitation*), Harold D Green and J Maxwell Little, *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest College*  
Peripheral vasodilator effect of a substance present in normal human urine
- 2 Vivian G Behrmann (*introduced by Robert Gesell*), *Dept of Laboratories, Henry Ford Hospital*  
Oxymograph studies during marked vaso motor changes
- 3 A B Hertzman and W C Randall, *Dept of Physiology, St Louis University Medical School*  
Further studies on the correlation between skin volume pulses and blood flow
- 4 K G Wakim, J C Terrier (*by invitation*) and E C Elkins (*by invitation*), *Mayo Clinic*  
The effects of percutaneous stimulation on the circulation in normal and in paralyzed extremities
- 5 G A Hallenbeck, E H Wood and O T Clagett (*by invitation*), *Section on Physiology and Division of Surgery, Mayo Clinic*  
Changes in radial arterial blood pressure during surgical resection of coarctation of the aorta
- 6 F Olmsted (*by invitation*), A C Corcoran, O Glasser (*by invitation*) and Irvine H Page, *Research Division of the Cleveland Clinic Foundation*  
Systolic pressure in the intact unanesthetized rat
- 7 R Lenel (*introduced by S Rodbard*), *Cardiovascular Dept, Research Institute, Michael Reese Hospital, Chicago*  
The secondary blood pressure rise and tachycardia occurring after the injection of epinephrine
- 8 D M Green, A D Johnson, A Lobb and G Cusick (*introduced by R Frederick Becker*), *University of Washington School of Medicine*  
The relation of initial blood pressure to adrenalectomy action
- 9 Kenneth E Jochim, *Dept of Physiology, University of Kansas*  
Arterial pulses simulated in electrical analogues of the circulatory system
- 10 Wilson C Grant (*introduced by Walter S Root*), *Dept of Physiology, College of Physicians and Surgeons, Columbia University*  
Oxygen in bone marrow blood during prolonged hemorrhagic anemia

- 11 **Hardin B Jones** (*introduced by Nello Pace*), *Division of Medical Physics, University of California*

Changes in tissue vascularity and inert gas exchange related to age

- 12 **V Winder and Mona M Anderson** (*by invitation*), *Research Laboratories, Park, Davis & Co, Detroit*

On the nature of tachyphylaxis and related phenomena

### PHYSIOLOGY C

Friday, March 19, 9 00 a m

ROOM 21, CONVENTION HALL

#### Adrenals

- 1 **Joseph T King, Carmen B Casas** (*by invitation*) and **Claire J Carr** (*by invitation*), *Dept of Physiology, University of Minnesota*  
Effect of corticotropin on ovariectomized C3H mice bearing adrenal adenomas

- 2 **Leon Hellman** (*by invitation*), **Raymond E Weston, Doris J W Escher** (*by invitation*) and **Louis Leiter** (*by invitation*), *Medical Division, Montefiore Hospital, New York*

The effect of adrenocorticotropin on renal hemodynamics and uric acid clearance

- 3 **Oscar Hechter and David Stone** (*by invitation*), *Worcester Foundation for Experimental Biology and Dept of Physiology, Tufts College Medical School*

In vitro action of adrenal cortical extract upon lymphocytes

- 4 **D J Ingle and M C Prestrud** (*by invitation*), *Research Laboratories, The Upjohn Company, Kalamazoo*

Changes in urinary glucose and nitrogen following adrenalectomy in the force-fed diabetic rat

- 5 **K E Paschkis, A Cantarow, A A Walkling** (*by invitation*), **W H Pearlman, A E Rakoff** (*by invitation*) and **D Boyle** (*by invitation*), *Jefferson Medical College*

Secretion and excretion of carbohydrate active adrenal compounds (oxysteroids)

- 6 **Gregory Pincus, Louise Romanoff** (*by invitation*) and **James Carlo** (*by invitation*), *Worcester Foundation for Experimental Biology*

Variations with age in neutral steroid excretion of men

- 7 **Hans Seyle, Pierre Haour** (*by invitation*) and **Claude Faribault** (*by invitation*), *Institut de Medecine et de Chirurgie Experimentales, Universite de Montreal*

Further studies concerning brain lesions induced by desoxy corticosterone overdosage in the rat

- 8 **Hudson Hoagland and David Stone** (*by invitation*), *Worcester Foundation for Experimental Biology*

Brain and muscle potassium in relation to stressful activities and adrenal cortical function

- 9 **Marguerite N Swift, Harvey M Patt and Ella B Tyree** (*introduced by Austin M Brues*), *Biology Division, Argonne National Laboratory*

The effect of adrenal cortical extract on adrenal response to total body X irradiation

- 10 **A Van Loo** (*by invitation*), **A Surtshin** (*by invitation*) and **L N Katz**, *Cardiovascular Dept, Research Institute, Michael Reese Hospital, Chicago*

The role of the adrenal in the arterial pressure responses to severe hypoxemia

- 11 **L Recant** (*by invitation*), **P H Forsham** (*by invitation*) and **G W Thorn**, *Dept of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital*

Effect of epinephrine on the circulating eosinophils

### PHYSIOLOGY D

Friday, March 19, 9 00 a m

ROOM 22, CONVENTION HALL

#### Special Senses

- 1 **C Haig and E M Haig** (*by invitation*), *Dept of Physiology and Biochemistry, New York Medical College*

The brightness threshold as a function of area and receptor number in various retinal regions

- 2 **E F MacNichol** (*by invitation*) and **H K Hartline**, *Johnson Research Foundation, University of Pennsylvania*

Responses to small changes of light intensity by the light adapted photoreceptor

- 3 **Hans-Lukas Teuber** (*by invitation*) and **Morris B Bender**, *Psychophysiological Laboratory, Dept of Neurology, New York University College of Medicine*

Critical flicker frequency in defective fields of vision

- 4 **George M Austin** (*by invitation*), **F H Lewey and Francis C Grant** (*by invitation*), *Laboratory of the Neurosurgical Service, Hospital of the University of Pennsylvania*

Physiological significance of preserved central vision in lesions of the optic tract vs optic radiation

- 5 **George Wald**, *Biological Laboratories, Harvard University*

Interconversions of retinene and vitamin A in vitro

- 6 Charles Sheard, *Division of Physics and Biophysical Research, Mayo Foundation and Mayo Clinic*  
Courses of dark adaptation and levels of vitamin A and carotene in normal and clinical conditions
- 7 Stanley K Davis (introduced by J W Heim), *Aero Medical Laboratory, Wright Field*  
Influence of intravenous cytochrome C upon visual acuity of the dark adapted human eye
- 8 William M Hart and Bruce F Chandler (by invitation), *Depts of Ophthalmology and Biochemistry, Temple University School of Medicine*  
Factors responsible for transmission of visible light by the fibrous tunic of the eye
- 9 J L Patterson, Jr and Ashton Graybiel (introduced by James V Warren), *Naval School of Aviation Medicine and Research, Pensacola, and Dept of Physiology, Emory University Medical School*  
A negative form of the oculo gravic illusion
- 10 Irving H Wagman, *Dept of Physiology, Jefferson Medical College*  
Pupil dilatation in darkness as effected by intensity and duration of pre exposure to white light
- 11 Freya Stone (by invitation) and Franz R Goetzel, *Dept of Medical Research, Permanente Foundation*  
Olfactory acuity and appetite effects of bitter tonics on olfactory acuity in normal human subjects
- 12 Elwood Henneman and Vernon Mountcastle (introduced by Philip Bard), *Dept of Physiology, Johns Hopkins University School of Medicine*  
Tactile localization in the thalamus of cat and monkey
- 3 William R Amberson, Rubert S Anderson (by invitation), Betty Chinn (by invitation) and T Erdos (by invitation), *Dept of Physiology, University of Maryland, and Institute of Physical Chemistry, University of Uppsala*  
Extraction of myogen and myosin from whole skeletal muscles
- 4 Julian M Tobias, *Dept of Physiology and Toxicity Laboratory, University of Chicago*  
Injury potential in potassium depleted frog muscle
- 5 Sheppard M Walker, *Dept of Physiology, Washington University School of Medicine*  
Action potentials induced by indirect stimulation of rat muscle after adrenalectomy, KCl treatment and tetanus
- 6 J S Denslow and David M Graham-Service (introduced by Irvin M Korr), *Still Memorial Research Trust, Kirksville*  
The spread of muscle action potentials from active to inactive areas
- 7 G Ling (introduced by R W Gerard), *Dept of Physiology, University of Chicago*  
Effect of stretch on membrane potential in frog muscle
- 8 Alexander Sandow, *Washington Square College of Arts and Sciences, New York University*  
Transverse latency relaxations of muscle stimulated with massive transverse shocks
- 9 H J Ralston, J R Close (by invitation), V T Inman (by invitation) and B Feinstein (by invitation), *Dept of Physiology, College of Physicians and Surgeons, San Francisco, and the University of California School of Medicine and College of Engineering*  
Dynamical and electrical features of human isolated voluntary muscle in isometric and isotonic contraction
- 10 Arthur W Martin and O M Sola (by invitation), *Dept of Physiology and Biophysics, University of Washington*  
The rate of atrophy of rat diaphragm
- 11 Ernest G Huf (introduced by Ernst Fischer), *Baruch Center of Physical Medicine and Dept of Physiology, Medical College of Virginia*  
Muscular atrophy as a state of "local hyperthyroidism"

### PHYSIOLOGY E

Friday, March 19, 9 00 a m

ROOM 17, CONVENTION HALL

#### Muscle

- 1 Ernst Fischer and Russel V Bowers (by invitation), *Baruch Center of Physical Medicine, Medical College of Virginia*  
Changes in zymohexase activity during denervation atrophy of skeletal muscle and their retardation by appropriate electrical treatment
- 2 William J Bowen and William E Poel (introduced by Heinz Specht), *Laboratory of Physical Biology, National Institute of Health, Bethesda*  
The effects of anoxia upon myoglobin concentration

### PHYSIOLOGY A

Friday, March 19, 1 45 p m

BALLROOM, CONVENTION HALL

#### Cerebral Cortex

- 1 Ernest Sachs, Jr and Samuel Brendler (introduced by John F Fulton), *Laboratory*

- of Physiology, Yale University School of Medicine  
Some effects of stimulation of the orbital surface of the frontal lobe in the dog and monkey
- 2 Robert B Livingston (by invitation) and John F Fulton, Laboratory of Physiology, Yale University School of Medicine  
"Cortical instability" a study of frequency effects
- 3 Wilder Penfield and Keasley Welch (by invitation)  
Instability of motor points and sensory points in the human cerebral cortex
- 4 W S McCulloch and Elwood Henneman, Dept of Psychiatry, University of Illinois  
The projection of area 19 to the reticular formation
- 5 Clinton N Woolsey and D H LeMessurier (by invitation), Dept of Physiology, Johns Hopkins University School of Medicine  
The pattern of cutaneous representation in the rat's cerebral cortex
- 6 Fred A Mettler and J Lawrence Pool (by invitation), Dept of Neurology, College of Physicians and Surgeons, Columbia University  
Removal of restral border of human area 4 followed by spasticity or lack of it
- 7 J E Ziegler and T W Rasmussen (introduced by Herbert Jasper), Dept of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute  
The effect of local graded pressure upon electrical activity and excitability of the motor cortex
- 8 Richard G Berry and Francis M Forster (introduced by M H F Friedman), Dept of Neurology, Jefferson Medical College  
Cerebral cortical effects of curare
- 9 John L Hampson (introduced by C N Woolsey), Dept of Physiology, Johns Hopkins University School of Medicine  
Relationships between the cerebral and cerebellar cortices in the cat
- 10 Herbert H Jasper and Jan Droogleever-Fortuyn (by invitation), Dept of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute  
Thalamo cortical systems and the electrical activity of the brain
- 11 Eli S Goldensohn (by invitation), Ewald W Dusse (by invitation), Joseph N Spencer (by invitation), William B Draper and Richard W Whitehead, Depts of Physiology and Pharmacology, and Psychiatry, University of Colorado Medical Center  
Studies on diffusion respiration VII Electrical cortical activity in dogs (Pharmacol)
- 12 Charles D Hendley (introduced by Horace W Davenport), Depts of Physiology and Pharmacology, University of Utah School of Medicine  
The effect of blood acid base changes on convulsive seizures

## PHYSIOLOGY B

Friday, March 19, 1 45 p m

ROOM 20, CONVENTION HALL

## Biophysics

- 1 F H Johnson, M B Baylor (by invitation) and D Fraser (by invitation), Dept of Biology, Princeton University  
The thermal denaturation of tobacco mosaic virus in relation to hydrostatic pressure
- 2 Harvey M Patt, Marguerite N Swift and Ella B Trève (introduced by Austin M Brues), Biology Division, Argonne National Laboratory  
Influence of temperature on radio sensitivity in the frog (*Rana pipiens*)
- 3 H O Parrack and D H Eldredge, Jr (introduced by H M Sweeney), Aero Medical Laboratory, Air Materiel Command, Wright Field  
Certain physiological reactions to intense sound fields
- 4 Alice M Stoll (by invitation), and James D Hardy, Russell Sage Institute of Pathology, The New York Hospital, and the Dept of Physiology, Cornell University Medical College  
Direct experimental comparison of several surface temperature measuring devices
- 5 Charles H Richards (by invitation) and James D Hardy, The New York Hospital and the Dept of Physiology, Cornell University Medical College  
An instrument for the measurement of total thermal radiation in the environment
- 6 Ronald Deering (introduced by A B Hertzman), Dept of Physiology, St Louis University Medical School  
The rod calorimeter
- 7 E E Painter and M C Moore (by invitation), Loyola University School of Medicine, Argonne National Laboratory, and University of Illinois College of Medicine  
Susceptibility of X rayed animals to histamine and to adenosine
- 8 Herbert Shapiro, Laboratory of Physical Biology, National Institute of Health, Bethesda  
The alteration in osmotically inactive fraction produced by cell activation

- 9 **Richard McFee** (*introduced by J S Robb*), *Dept of Pharmacology, Syracuse University College of Medicine*  
A critical discussion of the bioelectric "doublet" theory
- 10 **David F Waugh, M Janette Smith** (*by invitation*) and **Darthea F Fearing** (*by invitation*), *Dept of Biology, Massachusetts Institute of Technology*  
Regeneration of insulin fibrils with several reagents and the nature of the inter-insulin bond

### PHYSIOLOGY C

Friday, March 19, 1 45 p m

ROOM 21, CONVENTION HALL

#### Circulation and Heart

- 1 **Robert W Ramsey, George Fischer** (*by invitation*), and **Marie Louise Flinker** (*by invitation*), *Depts of Physiology and Pharmacology, Medical College of Virginia*  
The effect of eserine on the absolutely and relatively refractory period of turtle heart strips
- 2 **Frank L Pettinga** (*by invitation*) and **J W Stutzman**, *Dept of Pharmacology, Boston University School of Medicine*  
Effect of adrenalectomy or evisceration on cyclopropane induced cardiac arrhythmias in cats
- 3 **Ingrith J Deyrup** (*by invitation*) and **William W Walcott**, *Dept of Physiology, College of Physicians and Surgeons Columbia University*  
A study of the effects of the intravenous injection of hypertonic solutions on the heart rates of cats and dogs
- 4 **Augustus C P Bakos** (*introduced by Charles F Morgan*), *Dept of Physiology and Biophysics, Georgetown University School of Medicine*  
The maintenance of mean pulmonary arterial pressure after extreme right ventricular damage in the dog (Pharmacol)
- 5 **J Richard R Bobb, Donald C Kunze and William McCall, Jr** (*introduced by Harold D Green*), *Dept of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College*  
A method for production of cardiac infarction in the dog
- 6 **L H Peterson** (*introduced by H C Bazett*) and **G C Risman** (*introduced by H C Bazett*), *Dept of Physiology, School of Medicine, University of Pennsylvania*  
Direct blood pressure recording in man
- 7 **John R Smith and Masauki Hara** (*by invitation*), *Dept of Internal Medicine, Washington University School of Medicine*  
Experimental embolism of selected portions of pulmonary arterial bed
- 8 **Maurice B Visscher**, *University of Minnesota*  
The capacity changes in the pulmonary vascular bed with the respiratory cycle
- 9 **R L Riley** (*by invitation*), **A Himmelstein** (*by invitation*), **H L Motley** (*by invitation*), **H M Weiner** (*by invitation*) and **A Courmand**, *Cardio-Pulmonary Laboratory, Chest Service, Bellevue Hospital, and the Dept of Medicine, College of Physicians and Surgeons, Columbia University*  
Pulmonary circulation during exercise in normal individuals and in patients with chronic pulmonary disease
- 10 **Joseph H Hafkenschiel and James E Eckenhoff** (*introduced by Carl F Schmidt*), *Dept of Pharmacology and Harrison Dept of Surgical Research, School of Medicine, University of Pennsylvania*  
The oxygen content of coronary venous blood is affected by anoxia and cytochrome C (Pharmacol)
- 11 **Cecil K Drinker and Esther Hardenbergh** (*by invitation*), *Dept of Physiology, Harvard School of Public Health*  
Acute effects upon the lungs of dogs of large intravenous doses of alpha naphthyl thiouracil (ANTU)
- 12 **Harold Koenig** (*introduced by W F Windle*) and **Ruth Koenig** (*introduced by W F Windle*) *Anatomical Laboratories, University of Pennsylvania School of Medicine*  
Acute pulmonary edema produced by ammonium salts

### PHYSIOLOGY D

Friday, March 19, 1 45 p m

ROOM 22, CONVENTION HALL

#### Metabolism

- 1 **Francis X Fellers** (*by invitation*), **Henry L Barnett** (*by invitation*), **Helen McNamara** (*by invitation*) and **Kendrick Hare**, *The New York Hospital and Dept of Pediatrics, Cornell University Medical College*  
Decrease in the radiosodium and thiocyanate spaces during growth
- 2 **K I Altman, G W Casarett, R E Masters, T R Noonan, and K Salomon**, (*introduced by W F Bale*), *University of Rochester School of Medicine and Dentistry, Atomic Energy Project*  
Hemin synthesis with glycine containing  $C^{14}$  in its alpha-carbon atom
- 3 **John M Anderson**, (*introduced by Otto M Helff*), *Dept of Biology, Brown University*



Changes in nitrogen distribution in the Japanese beetle during metamorphosis

- 4 R Levine and N G Schneeberg (by invitation), Dept of Metabolism and Endocrinology, Research Institute, Michael Reese Hospital, Chicago

The disposal of intravenously administered amino acids by normal, hepatectomized, depancreatized and hyperthyroid dogs

- 5 C Martin Rhode (by invitation), William M Parkins and Harry M Vars, Harrison Dept of Surgical Research, Schools of Medicine, Univ of Pennsylvania

Nitrogen balances of dogs continuously infused with 50% glucose and protein preparations

- 6 L Van Middlesworth and D H Copp (introduced by J P Quigley), Division of Physiology, University of California, and University of Tennessee

Post fracture nitrogen loss with and without methionine supplements to good and poor protein diets

- 7 Jay A Smith (by invitation), Piero P Foa, Harriet R Weinstein (by invitation), A Sidney Ludwig (by invitation), and J Marvin Wertheim (by invitation), Dept of Physiology and Pharmacology, Chicago Medical School

Some toxic effects of acid and neutralized thiamine solutions

- 8 Isaac M Berry (by invitation) and A C Ivy, Dept of Clinical Science, University of Illinois College of Medicine

The tolerance of dogs to intravenously administered fatty chyle and synthetic fat emulsion

- 9 Piero P Foa and Harriet R Weinstein (by invitation), Dept of Physiology and Pharmacology, Chicago Medical School

The lipids of the rat brain in choline deficiency

- 10 M H Hack (introduced by Hubert R Catchpole), Dept of Pathology, University of Illinois College of Medicine

The phosphatide composition of human erythrocytes

- 11 Wiktor W Nowinski (introduced by C M Fomerat), Tissue Culture Laboratory and the Psychopathic Hospital, Medical Branch, University of Texas

Influence of anti organ sera upon the oxygen uptake of the spleen and brain

- 12 J H Peters (by invitation), L Greenman (by invitation), and T S Danowski, Dept of Research Medicine and the Renziehausen Foundation University of Pittsburgh School of Medicine

Beneficial effects of calcium chloride in fluoride poisoning

## PHYSIOLOGY E

Friday, March 19, 1 45 p m

ROOM 17, CONVENTION HALL

### Blood Volume

- 1 J LaRue Wiley (introduced by H C Bazett), Michael Newton (introduced by H C Bazett) and J B Tracy, Jr (introduced by H C Bazett), Dept of Physiology, Medical School, University of Pennsylvania

Relationship in the dog between inferior vena caval pressure and total plasma protein

- 2 C H William Ruhe and Paul L McLain (introduced by C C Guthrie), Dept of Physiology and Pharmacology, School of Medicine, University of Pittsburgh

A comparison of heat coagulable serum solids with serum protein determined by a Kjeldahl method

- 3 J P Henry, H Jacobs (by invitation), H Meeham (by invitation) and A Karstens (by invitation), Dept of Physiology, University of Southern California, and Aero Medical Lab, Air Materiel Command, Wright Field

The effect of pressure breathing on circulating blood volume. (1) Fluid loss into the tissue spaces

- 4 Thomas H Allen and Peter D Orahovats (by invitation), Dept of Physiology, College of Physicians and Surgeons, Columbia University

A method for estimating traces of T 1824 by combination with cellophane

- 5 Robert T Nieset (introduced by H S Mayer-son), School of Medicine, Tulane University, and the Research Division of the Alton Ochsner Medical Foundation

Blood volume determination by plasma dye dilution and dilution of red cells tagged with P 32

- 6 A H Tuttle (introduced by R R Overman), Dept of Physiology, University of Tennessee College of Medicine

Blood, plasma and extracellular fluid volumes in experimental low colonic obstruction

- 7 Paul L McLain and C H William Ruhe (introduced by C C Guthrie), Dept of Physiology and Pharmacology, School of Medicine, University of Pittsburgh

On estimating the relative volumes of corpuscles and serum in blood

- 8 James C Moore and O W Shadle (introduced by Hampden C Lawson), Dept of Physiology, University of Louisville School of Medicine

Estimation of the total circulating red cell volume by the use of methemoglobin-tagged cells

- 9 O W Shadle and James C Moore (introduced by Hampden C Lawson), *Dept of Physiology, University of Louisville School of Medicine*

Measurement of red cell volume loss by the use of methemoglobin-tagged cells

- 10 R L Post (by invitation) and C R Speakman, *Dept of Physiology, University of Pennsylvania Medical School*

Variation of total circulating hemoglobin and reticulocyte count with season and following hemorrhage

## PHYSIOLOGY

### Papers Read by Title

- 1 E F Adolph, *Dept of Physiology, University of Rochester*

Cold tolerance and cold immersion in infant rats

- 2 Grant R Bartlett (introduced by William G Clark), *Scripps Metabolic Clinic, La Jolla*  
Molecular structure and activity of "vitamin P"-like substances Inhibition of succinoxidase

- 3 James H Birnie (by invitation), W J Eversole (by invitation) and Robert Gaunt, *Dept of Zoology, Syracuse University*

Survival of adrenalectomized-nephrectomized rats treated with desoxycorticosterone

- 4 Stanley Block (by invitation), Louis Rosenberg (by invitation), R H Broh-Kahn (by invitation) and I Arthur Mirsky, *May Institute for Medical Research of the Jewish Hospital, Cincinnati*

The source of uropepsin in man

- 5 William J Bowen (introduced by Heinz Specht), *Laboratory of Physical Biology, National Institute of Health, Bethesda*

Alkali decomposition of myoglobin

- 6 Daniel A Brody (by invitation), J D Lawson (by invitation) and J P Quigley, *Dept of Physiology, University of Tennessee*

Production of waves of inhibition in the esophageal-fundic region

- 7 F Brown (by invitation) and S Rodbard, *Cardiovascular Dept, Research Institute, Michael Reese Hospital, Chicago*

Pulmonary arterial pressure of the chicken

- 8 L D Carlson, A W Martin, and V Gattone (by invitation), *Dept of Physiology and Biophysics, School of Medicine, University of Washington*

An apparatus for the measurement of pulmonary function

- 9 T S Chang (by invitation) and Smith Freeman, *Dept of Experimental Medicine, Northwestern University and Division of Experimental Medicine, Mayo Foundation*

Changes in blood level of citric acid and calcium in nephrectomized dogs

- 10 John L Chapin (by invitation) and Hermann Rahn, *Dept of Physiology, University of Rochester School of Medicine and Dentistry*  
Effect of CO<sub>2</sub> upon the ventilation response of the beaver

- 11 George P Child (by invitation), B S Hardman (by invitation), R A Woodbury and R Torpin (by invitation), *Depts of Pharmacology and Obstetrics and Gynecology, University of Georgia School of Medicine*

Ephedrine effect on human uterine contractions

- 12 William G Clark and T A Geissman (by invitation), *Scripps Metabolic Clinic, La Jolla, and Dept of Chemistry, University of California*

Molecular structure and activity of "vitamin P" like substances Inhibition of oxidation of epinephrine

- 13 J M Crismon, *Dept of Physiology, Stanford University School of Medicine*

Errors induced by phosphate in flame photometer analysis of tissue ash for sodium and potassium

- 14 C G Drake (by invitation) and G W Starvaky, *Dept of Physiology, Faculty of Medicine, University of Western Ontario*

Effect of convulsant agents on partially isolated neurons of the central nervous system

- 15 Janet Fairfield (introduced by E F Adolph), *Dept of Physiology, University of Rochester*

Effects of cold on infant rats

- 16 A M Freedman, (by invitation) and H E Himwich, *Medical Division, Army Chemical Center, Md*

Effect of size, sex and pregnancy on the lethality of di-isopropyl fluorophosphate (DFP)

- 17 M Friedman and S O Byers (by invitation), *The Harold Brunn Institute for Cardiovascular Research, Mt Zion Hospital, San Francisco*

Comparison of the renal clearances of allantoin and inulin in man

- 18 M H F Friedman and J E Thomas, *Dept of Physiology, Jefferson Medical College*

Preparation and assay of secretin

- 19 Frederick A Fuhrman, *Dept of Physiology, Stanford University School of Medicine*

Effect of rutin in experimental frostbite

- 20 R G Grenell, B Moore (by invitation), H S Burr (by invitation), W Brown (by invitation) and S Friedman (by invitation), *Divisions of Neuroanatomy and Psychiatry, Yale University School of Medicine and Fairfield State Hospital, Newtown, Conn*  
Electrical correlates of psychiatric disturbances

- 21 R E Gosselin (introduced by E E Adolph),  
*Dept of Physiology, University of Rochester*  
Acute hypothermia in guinea pigs
- 22 Richard A Groat, *Dept of Anatomy, Bowman Gray School of Medicine of Wake Forest College*  
Relationship of volumetric rate of blood flow to arterial diameter
- 23 C C Guthrie and Marian E Lee (by invitation), *Dept of Physiology and Pharmacology, School of Medicine, University of Pittsburgh*  
Quantitative effect of hemorrhage on plasma proteins
- 24 Frederick S Hammett, *The Lankenau Hospital Research Institute, Philadelphia*  
Possible influence of cosmic energy cycles on growth
- 25 William A Hiestand and Forst D Fuller (by invitation), *Laboratory of Animal Physiology, Purdue University, and Dept of Zoology, DePauw University*  
Is heat death due to a myotoxic factor?
- 26 Robert Hodes, *Dept of Physical Medicine, Graduate School of Medicine, and the Eldridge Reeves Johnson Foundation, University of Pennsylvania*  
Muscle action potentials in human poliomyelitis before and after treatment by Billig's method
- 27 S M Horvath, R N Miller (by invitation) and B K Hutt (by invitation), *Dept of Physical Medicine, Graduate School of Medicine, University of Pennsylvania*  
Heating of human muscle tissue by micro waves
- 28 C Riley Houck, *Depts of Physiology, New York University College of Medicine and the University of Tennessee College of Medicine*  
Statistical analysis of filtration rate and renal plasma flow in normal dog and man
- 29 O H Janton (by invitation), H P Redondo (by invitation) and J C Scott, *Dept of Physiology, The Hahnemann Medical College*  
Pulmonary gas exchange following ligation of a pulmonary artery in man
- 30 E Kaplan and N R Joseph (introduced by C I Reed), *Dept of Physiology, College of Medicine and Department of Chemistry, College of Pharmacy, University of Illinois*  
Determination of circulation rate in articular structures
- 31 Elizabeth N King (by invitation), M H F Friedman and I J Pincus (by invitation), *Dept of Physiology, Jefferson Medical College, Philadelphia*  
Tryptic activity of pancreatic juice after dilution with gastric juice
- 32 Bruno Kisch, *Marine Biological Laboratory, Woods Hole*  
The electrogram of the fish heart
- 33 Alfred Leimdorfer, *Dept of Pharmacology, University of Illinois College of Medicine*  
Electroencephalographic analysis of action of amide, morphine and strychnine on the central nervous system
- 34 D H LeMessurier (introduced by C N Woolsey), *Dept of Physiology, Johns Hopkins University School of Medicine*  
Auditory and visual areas of the cerebral cortex of the rat
- 35 Richard W Lippman (introduced by T Addis), *Dept of Medicine, Stanford University School of Medicine*  
Effects of protein and fluid consumption upon plasma volume and circulating protein in the rat
- 36 David I Macht, *Division of Pharmacology, Laboratories of Sinai Hospital, Baltimore*  
Thromboplastic effects of sulfa and penicillin combinations
- 37 Martin B Macht and Elizabeth L Pillion (introduced by H S Belding), *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass*  
Changes in skin temperature and blood flow of hand following ingestion of certain amino acids
- 38 N S R Maluf, *Dept of Pharmacology, University of Louisville School of Medicine and the Louisville General Hospital*  
Urea clearance by perfusion of the entire intact small intestine in man
- 39 John Marshall (by invitation) and G E Wackerlin, *Dept of Physiology, University of Illinois College of Medicine*  
Purification of renin
- 40 E M Martin (by invitation), M J Martin (by invitation) J P Henry, J L Gamble, Jr (by invitation) and R S Shaw (by invitation), *Aero Medical Laboratory, Air Materiel Command, Wright Field*  
The effects of various pressure application devices (anti g suits) upon arterial and venous pressures
- 41 A T Milhorat, *Depts of Psychiatry and Medicine, Cornell University Medical College, The Russell Sage Institute of Pathology and the New York Hospital*  
Effect of delta- and gamma tocopherol on creatinuria in progressive muscular dystrophy
- 42 Dorothy Nelson (introduced by J S Gray), *Dept of Physiology, Northwestern University Medical School*  
The sodium chloride tolerance of female mice
- 43 John F Perkins, Jr and M C Li (introduced

- by E M Landis), *Dept of Physiology, Harvard Medical School*  
The effects of changing the temperature of the normal and denervated micturating membrane
- 44 J J Reinhard, Jr (by invitation) O Glasser (by invitation) and Irvine H Page, *Research Division of the Cleveland Clinic Foundation*  
Effects of hemorrhagic shock on hepatectomized and bilaterally nephrectomized-hepatectomized dogs
- 45 Boris B Rubenstein, *Dept of Metabolism-Endocrinology Research, Michael Reese Hospital*  
Does progesterone or testosterone alter the human vaginal smear?
- 46 Gerald R Seaman (introduced by C G Wilber) *Biological Laboratory, Fordham University*  
Relation between growth and synthesis of lipids in colpidium
- 47 Herbert Shapiro, *Laboratory of Physical Biology, National Institute of Health, Bethesda*  
The retarding action of vitamin C in physiological concentrations on the rate of cell division
- 48 Walter B Shelley and Frank N Melton (by invitation), *Dept of Dermatology, University of Pennsylvania School of Medicine*  
The permeability of normal human skin to histamine
- 49 Ephraim Shorr, B W Zweifach and S Baez (by invitation), *Dept of Medicine, Cornell University Medical College and the New York Hospital*  
Hepato-renal factors in circulatory homeostasis
- 50 Franklin F Snyder, *Harvard Medical School*  
Oxygenation of the cord blood of breathing fetuses during a prolonged period
- 51 F J Sichel and Cheryl Parkhurst (by invitation), *University of Vermont College of Medicine*  
The leakage of potassium from injured muscle fibres
- 52 Verne W Swigert (by invitation), Hamilton R Fishback (by invitation), Lillian E Cisler (by invitation) and Frederic T Jung, *Northwestern University Medical School*  
Silicone implantations in the rat
- 53 Julian M Tobias, *Dept of Physiology and Toxicity Laboratory, University of Chicago*  
Sodium and potassium in insects larvae, pupae and adults
- 54 Clara Torda and Harold G Wolff, *New York Hospital and Depts of Medicine (Neurology) and Psychiatry, Cornell University Medical College*  
Effect of acetylcholine, caffeine and alkaloids on activity of muscle adenosinetriphosphatase
- 55 Frank Visscher, *Research Laboratories, Upjohn Co, Kalamazoo*  
Fractionation studies of enterogastrone activity in pyloric ligation rats
- 56 G van Wageningen, *Dept of Obstetrics and Gynecology, Yale University School of Medicine*  
Vitamin A deficiency in pregnancy
- 57 Virginia Weimar (by invitation) and Rosalind Wulzen, *Dept of Zoology, Oregon State College*  
Leucocytosis of guinea pigs deficient in the anti stiffness factor
- 58 Arne N Wick and Francis Pauls (introduced by Irlton N Mackay), *Scripps Metabolic Clinic, La Jolla*  
Preparation of anti ulcer substance from bovine urine
- 59 Charles G Wilbur, *The Biological Laboratory, Fordham University*  
Glucose metabolism in marine invertebrates
- 60 D L Wilson (by invitation) and J F Manery, *Dept of Biochemistry, University of Toronto*  
Sodium, potassium and chloride in leucocytes
- 61 Claude V Winder, Mona M Anderson (by invitation) and Herve C Parke (by invitation), *Research Laboratories, Parke, Davis & Co, Detroit*  
Comparative properties of six phenethylamines
- 62 Verner J Wulff (by invitation) and Theodore L Jahn, *Dept of Zoology and Physiology, University of Illinois, and Depts of Physiology and Zoology, State University of Iowa*  
Relation of retinal and optic nerve response to intensity of illumination of the grass hopper eye
- 63 B W Zweifach, S Baez (by invitation), R F Furchgott (by invitation) and Ephraim Shorr, *Dept of Medicine, Cornell University Medical College and the New York Hospital*  
Hepato-renal factors in circulatory homeostasis

## AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC.

## THIRTY-NINTH ANNUAL MEETING

## BIOCHEMISTRY A

Tuesday, March 16, 9 00 a m

ROOM C, CONVENTION HALL

## Acetate Metabolism

- 1 Albert L Lehninger and Eugene P Kennedy (by invitation), Depts of Surgery and Biochemistry, University of Chicago  
The fatty acid oxidase complex of rat liver and intracellular structures
- 2 Nathan O Kaplan (by invitation) and Fritz Lipmann, Biochemical Research Lab, Massachusetts General Hospital, and the Dept of Biological Chemistry, Harvard University Medical School, Boston  
Reactions between acetate, acetyl phosphate and the adenylic acid system in tissue and bacterial extracts
- 3 Harold Strecker (by invitation), L O Krampitz, and Harland G Wood, Dept of Biochemistry, Western Reserve University School of Medicine, Cleveland  
The role of acetylphosphate in the phosphoclastic and dismutation reactions of pyruvate
- 4 David Novelli (by invitation) and Fritz Lipmann, Biochemical Research Lab, Massachusetts General Hospital, and the Dept of Biological Chemistry, Harvard University Medical School, Boston  
Respiratory metabolism in yeast and coenzyme A levels Effect of phenyl panthenone on coenzyme A synthesis
- 5 Michael S Weiss, Helen Vorapaeff, and David Nachmansohn (introduced by H T Clarke), Dept of Neurology, College of Physicians and Surgeons, Columbia University, New York  
Inhibitors of choline acetylase
- 6 Morris Soodak (by invitation) and Fritz Lipmann, Biochemical Research Lab, Massachusetts General Hospital, and the Depts of Biological Chemistry and Medicine, Harvard University Medical School, Boston  
An enzymatic micro-method for determination of acetic acid
- 7 Simon Black (introduced by E S Guzman Barron), Chemical Div, Dept of Medicine, University of Chicago  
A microanalytical method for acetic and other volatile acids
- 8 Konrad Bloch and W Kramer (by invitation), Dept of Biochemistry and Inst of Radio-

biology and Biophysics, University of Chicago

Synthesis of fatty acids in rat liver slices

- 9 H S Anker (introduced by E A Evans, Jr), Dept of Biochemistry, University of Chicago  
On the fate of labeled pyruvic acid in the intact animal
- 10 R Gordon Gould (by invitation), I M Rosenberg (by invitation), Marott Sinex (by invitation), and A Baird Hastings, Dept of Biological Chemistry, Harvard University Medical School, Boston  
Rate of  $C^{14}O_2$  excretion following intraperitoneal administration of isotopic bicarbonate and acetate
- 11 W D Armstrong, Jack Schubert (by invitation), and Arthur Lindenbaum (by invitation), Dept of Physiological Chemistry, University of Minnesota Medical School, Minneapolis  
Tissue incorporation and excretion of radioactive carbon administered as carbonate

## BIOCHEMISTRY B

Tuesday, March 16, 9 00 a m

ROOM D, CONVENTION HALL

## Amino Acid Determinations

- 1 Stanford Moore (by invitation) and William H Stein, The Rockefeller Institute for Medical Research, New York  
Chromatography of amino acids Colorimetric ninhydrin method for analysis of the effluent
- 2 William H Stein and Stanford Moore (by invitation), The Rockefeller Institute for Medical Research, New York  
Chromatography of amino acids Solvent systems for the fractionation of protein hydrolysates
- 3 Albert S Keston (by invitation), Sidney Udenfriend (by invitation), and R Keith Cannan, Dept of Chemistry, New York University College of Medicine  
Application of the isotopic derivative method of analysis to protein hydrolysates
- 4 Milton Levy and Evelyn Slobodiansky (by invitation), Dept of Chemistry, New York University College of Medicine  
The arrangement of amino acids in silk, an application of the isotopic derivative technique

- 5 **Albert S Keston** (*by invitation*), **Sidney Udenfriend** (*by invitation*), and **Milton Levy**, *Dept of Chemistry, New York University College of Medicine, New York*  
Quantitative analysis of protein hydrolysates on paper chromatograms by means of the isotopic derivative method
- 6 **N F Young** and **F Homburger** (*introduced by T F Gallagher*), *The Sloan-Kettering Inst for Cancer Research, Research Div Memorial Cancer Center, New York*  
The application of paper chromatography to the study of aminoaciduria in patients with liver disease
- 7 **Stanley R Ames** and **Hugh A Risley** (*introduced by P L Harris*), *Labs of Distillation Products, Inc, Rochester*  
Aminoaciduria in progressive muscular dystrophy
- 8 **J Logan Irvin** and **Elinor Moore Irvin** (*by invitation*), *Dept of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore*  
The determination of various amines by proton exchanges with ethyl eosin in non-aqueous solvents
- 9 **M X Sullivan**, *Chemo Medical Research Inst, Georgetown University, Washington, D C*  
A comparison of reactions of cysteine and Beta-beta dimethyl cysteine
- 10 **Dean Burk**, **Silvio Fiala** (*by invitation*), **John Hearon** (*by invitation*), **Marie Hesselbach** (*by invitation*), **Milton Levy** (*by invitation*), and **Arthur L Schade**, *National Cancer Institute, National Institute of Health, Bethesda, Md, and Overly Biochemical Research Foundation, New York*  
Reversible oxygenation, irreversible oxidation, and decarboxylation of cobalt amino acid complexes with oxygen gas
- 11 **Martin E Hanke**, *Dept of Biochemistry, University of Chicago*  
Conditions necessary for complete decarboxylation of pure L lysine and L tyrosine by amino decarboxylases
- 2 **Erland C Gjessing** (*introduced by Alfred Chanutin*), *Biochemical Lab, University of Virginia, Richmond*  
Fractionation of lymphoid tissues
- 3 **Adolph Abrams** (*by invitation*) and **Philip P Cohen**, *Dept of Physiological Chemistry, University of Wisconsin Medical School, Madison*  
Fractionation studies on the cytoplasmic proteins of human lymphoid tissue
- 4 **Jordi Folch** and **L L Uzman** (*by invitation*), *McLean Hospital, Waverley, Mass, and Harvard University Medical School, Boston*  
Brain proteins isolation of a birefringent liponucleoprotein
- 5 **J Murray Luck** and **A Clark Griffin** (*by invitation*), *Dept of Chemistry, Stanford University, Calif*  
Fractionation of liver proteins
- 6 **Frank W Putnam**, **Lloyd M Kozloff** (*by invitation*), and **E A Evans, Jr**, *Dept of Biochemistry, University of Chicago*  
Purification and properties of *E coli* bacteriophage T<sub>2</sub>
- 7 **D W Woolley**, *Labs of The Rockefeller Institute for Medical Research, New York*  
Some crystalline peptides isolated from enzymic digests of insulin and their relationship to streptogenin
- 8 **Frank A Csonka**, *Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U S Dept of Agriculture, Washington*  
Dietary influence on the amino acid composition of proteins
- 9 **T L McMeekin**, **B D Polis** (*by invitation*), **E S Della Monica** (*by invitation*), and **J H Custer** (*by invitation*), *Eastern Regional Research Lab, Philadelphia*  
A crystalline compound of  $\beta$ -lactoglobulin with dodecyl sulfate
- 10 **Stephan Ludewig** and **Alfred Chanutin**, *Biochemical Lab, University of Virginia, Charlottesville*  
Studies on the agglutination of human and rat red cells by castor bean extracts

### BIOCHEMISTRY C

Tuesday, March 16, 9 00 a m

Room B, CONVENTION HALL

#### Proteins

- 1 **Sidney Roberts** (*by invitation*) and **Abraham White**, *Dept of Physiological Chemistry, Yale University School of Medicine, New Haven*  
Biochemical characterization of lymphoid tissue proteins

### BIOCHEMISTRY D

Tuesday, March 16, 9 00 a m,

Room S, CONVENTION HALL

#### Lipids

- 1 **Walter Marx** and **Mortimer Lipsett** (*introduced by Harry J Deul, Jr*), *Dept of Biochemistry, University of Southern California Medical School, Los Angeles*

- Enzymatic destruction of cholesterol by rat liver extract *in vitro*
- 2 R T Holman (introduced by P B Pearson), *Medicinska Nobelinst, Stockholm, and Dept of Biochemistry and Nutrition, A and M College of Texas, College Station*  
Coupled oxidations in enzymatically oxidized linoleic acid
  - 3 Camillo Artom and Marjorie A Swanson (by invitation), *Dept of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston Salem*  
On the absorption of phospholipides
  - 4 Camillo Artom and W E Cornatzer (by invitation), *Dept of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston Salem*  
The action of ethanolamine, monomethyl- and dimethyl ethanolamine on lipid phosphorylation
  - 5 Warren M Sperry and Florence C Brand (by invitation), *Dept of Biochemistry, New York State Psychiatric Institute, New York*  
The determination of choline in brain lipids
  - 6 Sie Hsien-gieh (by invitation), C E Faust (by invitation), and Harold H Williams, *Dept of Biochemistry, Cornell University, Ithaca*  
Studies on cerebrosides
  - 7 Alfred E Koehler, Elsie Hill (by invitation), and Frank Fearney (by invitation), *Santa Barbara Cottage Hospital and The Sansum Clinic Research Foundation, Santa Barbara*  
Studies on the micro molecular distillation of blood lipids
  - 8 J C Forbes and William B Porter (by invitation), *Depts of Biochemistry and Medicine, Medical College of Virginia, Richmond*  
Fractionation of serum cholesterol

## JOINT SESSION OF THE FEDERATION

Tuesday, March 16, 1 30 p m

BALLROOM, CONVENTION HALL

Program on page 314

## BIOCHEMISTRY BUSINESS MEETING

Tuesday, March 16, 4 15 p m

ROOM C

## BIOCHEMISTRY A

Wednesday, March 17, 9 00 a m

ROOM C, CONVENTION HALL

## Phosphorus Compounds

- 1 Otto Meyerhof and Peter Oesper (by invitation), *Dept of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia*  
The free energy of phosphorylation
- 2 Ernst Vischer (by invitation) and Erwin Chargaff, *Dept of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*  
Studies on the composition of nucleic acids
- 3 Gerhard Schmidt, Ricardo Cubiles (by invitation), and S J Thannhauser, *Boston Dispensary, Tufts College Medical School*  
Ribopolynucleotide fractions formed during the enzymatic hydrolysis of yeast ribonucleic acid
- 4 Zacharias Dische, *Dept of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*  
The breakdown of free and combined adenosine 5 phosphate (AdPh) in intact human erythrocytes
- 5 E Racker (introduced by S Ochoa), *Dept of Bacteriology, New York University College of Medicine*  
Enzymatic formation and breakdown of pentose phosphate
- 6 W Wayne Kielley (introduced by Otto Meyerhof), *Dept of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia*  
A new adenosinetriphosphatase of muscle
- 7 Arthur Kornberg and Olov Lindberg (introduced by Carl F Cori), *Dept of Biological Chemistry, Washington University School of Medicine, St Louis*  
DPN pyrophosphatase
- 8 Aser Rothstein and Rebecca Meier (introduced by Harvey B Haag), *University of Rochester School of Medicine and Dentistry, N Y*  
Adenylpyrophosphatases and other phosphatases in the cell surface of living yeast (Pharmacol)
- 9 H G Albaum, M Ogur (by invitation), H Mendelow (by invitation), and A Hirschfeld (by invitation), *Depts of Biology and Chemistry of Brooklyn College*  
Adenine-pentose pyrophosphate from mung beans
- 10 Oscar Bodansky and Norma Strachman (by invitation), *Dept of Pharmacology, Cornell University Medical College, New York*  
The inhibitory effects of  $\alpha$  amino acids on phosphatase activity
- 11 Eunice V Flock and Jesse L Bollman, *Mayo Foundation, Rochester, Minn*  
Alkaline phosphatase activity of intestinal lymph of the rat

## BIOCHEMISTRY B

Wednesday, March 17, 9 00 a m  
Room D, CONVENTION HALL

## Amino Acid Metabolism

- 1 **H R Crookshank** (by invitation) and **Clarence P Berg**, *Biochemistry Dept, Medical College of Alabama, Birmingham, and the Biochemical Lab, State University of Iowa, Iowa City*  
Changes in the composition of the blood of rats fed L histidine
- 2 **Ella H Fishberg**, *Biochemical Lab of Beth Israel Hospital, New York*  
The quinoid stage of tyrosine metabolism
- 3 **W Knowlton Hall** (by invitation), **V P Sydenstricker** and **Katrine Rawls** (by invitation), *Depts of Medicine and of Biochemistry, University of Georgia School of Medicine, Augusta*  
Factors affecting the excretion of metabolites of phenylalanine and tyrosine in alkaptanuria (Nutrition)
- 4 **Halvor N Christensen**, *The Children's Hospital, and the Dept of Biological Chemistry, Harvard University Medical School, Boston*  
The distribution of amino acids between fetal and maternal extracellular fluids and cells
- 5 **Philip Handler**, **Henry Kamin** (by invitation), and **Jerome S Harris** (by invitation), *Depts of Biochemistry and Pediatrics, Duke University School of Medicine, Durham*  
The metabolism of parenterally administered amino acids I Glycine
- 6 **David Shemin**, **Irving M London** (by invitation), and **D Rittenberg**, *Depts of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York*  
The *in vitro* synthesis of heme from glycine by the nucleated red blood cell
- 7 **Irving M London** (by invitation), **Randolph West**, **David Shemin**, and **D Rittenberg**, *Depts of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, and the Presbyterian Hospital in the City of New York*  
On the origin of stercobilin in humans
- 8 **Louis D Greenberg** and **James F Rinehart** (by invitation), *Dns of Pathology and Pharmacology, University of California Medical School, San Francisco*  
Xanthurenic acid excretion in pyridoxine deficient rhesus monkeys
- 9 **Jack Schultz** and **George T Rudkin** (introduced by Gerit Toennies), *Lankenau Hospital Research Inst, and Inst for Cancer Research, Philadelphia*  
Absence of a sparing action of tryptophane on nicotinamide requirements of the fly, *Drosophila melanogaster*

- 10 **Robert MacVicar** (by invitation) and **R H Burris**, *Dept of Biochemistry, University of Wisconsin, Madison*  
Studies on the nitrogen metabolism of tomato using N<sup>15</sup>-labeled ammonium sulfate

- 11 **James M Orten**, *Dept of Physiological Chemistry, Wayne University College of Medicine, Detroit*  
Further studies on the effect of cysteine and histidine on the production of cobalt polycythemia

## BIOCHEMISTRY C

Wednesday, March 17, 9 00 a m  
Room B, CONVENTION HALL

## Liver Protein Regeneration, Nitrogen Balance

- 1 **D Rittenberg**, **Edith E Sproul** (by invitation), and **David Shemin**, *Dept of Biochemistry and Pathology, College of Physicians and Surgeons, Columbia University, New York*  
Rate of protein formation in the livers of partially hepatectomized rats
- 2 **Harry M Vars** and **Charles E Friedgood** (by invitation), *Harrison Dept of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia*  
The influence of fat in the diet upon nitrogen metabolism and liver protein regeneration
- 3 **Leon L Miller**, *Dept of Biochemistry, Jefferson Medical College, Philadelphia*  
Loss and restoration of rat liver enzymes (activity) related to dietary changes in liver protein
- 4 **Sam Sifter**, **David M Harkness** (by invitation), **B Feldman** (by invitation), **Leonard Rubin** (by invitation), and **Edward Muntwyler**, *Dept of Biochemistry, Long Island College of Medicine, Brooklyn*  
Concentration changes of certain vitamins and enzymes in livers of rats on protein free diet
- 5 **Carl Alper** (by invitation), **Shirley DeBiase** (by invitation), and **Bacon F Chow**, *Div of Protein Chemistry, The Squibb Inst for Medical Research, New Brunswick*  
The rate of disappearance of human albumin in normal and protein depleted dogs
- 6 **W C Hess**, *Georgetown Medical School, Washington*  
The rates of absorption of L- and DL methionine
- 7 **James D Solomon** and **Stanley W Hier** (introduced by Olaf Bergeim), *Dept of Biological Chemistry, University of Illinois College of Medicine, and the Research Labs, The Wilson Labs, Chicago*  
Influence of feeding amino acids on the spinal fluid level of free amino acids



- 8 Robert H Silber, E E Howe, and Curt C Porter (introduced by Edgar G Miller), *Merck Inst for Therapeutic Research and the Merck Research Labs, Rahway, N J*

The urinary excretion of amino acids and peptides after intravenous infusion to dogs

- 9 H E Sauberlich (by invitation) and C A Baumann, *Dept of Biochemistry, College of Agriculture, University of Wisconsin, Madison*

Amino acid excretion as influenced by dietary proteins of different biological value

- 10 George S Samuelsen (by invitation), Grace E Griffin (by invitation), Lois E Griffith (by invitation), Sam Seifter and Edward Muntwyler, *Dept of Biochemistry, Long Island College of Medicine, Brooklyn*

Blood and "tissue" protein changes in dogs on protein deficient diets with and without supplementation

### BIOCHEMISTRY D

Wednesday, March 17, 9 00 a m

ROOM S, CONVENTION HALL

#### Steroids

- 1 H Hirschmann, Frieda B Hirschmann (by invitation), and Margaret A Daus (by invitation), *Dept of Medicine Western Reserve University and the Lakeside Hospital, Cleveland*

Stereochemistry of allopregnanetriol 3,16,20 of mare's urine

- 2 William J Haines, Mari P Goodwin, George Pish, and Foil A Miller (introduced by Marvin H Kuizenga), *Research Labs, The Upjohn Co, Kalamazoo, Mich, and Div of Physical Chemistry, University of Illinois, Urbana*

An infra-red and x ray study of polymorphism in 5 pregnen 3( $\beta$ )ol 20 one

- 3 W H Pearlman *Dept of Biochemistry, Jefferson Medical College of Philadelphia*

The isolation of pregnanol 3( $\alpha$ ) one 20, comp'd Y ( $C_{27}H_{46}O_2$ ) and comp'd Z ( $C_{27}H_{46}O_2$ ) from the bile of pregnant cows

- 4 Seymour Lieberman (by invitation), David K Fukushima (by invitation), and Konrad Dobriner, *Sloan Kettering Inst for Cancer Research*

Adrenal cortical metabolites in human urine

- 5 Benjamin F Stummel *Rees Stealy Medical Research Fund, San Diego*

The effect of zinc-hydrochloric acid hydrolysis on the estrogens in human urine

- 6 Harold J Nicholas (by invitation), Sidney A Thayer, and Edward A Doisy, *Lab of Bio-*

*logical Chemistry, St Louis University School of Medicine*

Estrogenic activity of doisy nolic acid and related compounds

- 7 Ernest J Umberger and Jack M Curtis (introduced by Arnold J Lehman), *Div of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C*

The estimation of alpha and beta estradiols and of estrone and equilin in binary mixtures (Pharmacol)

- 8 Blaine H Levedahl (by invitation) and Leo T Samuels, *Dept of Biochemistry, University of Utah School of Medicine, Salt Lake City*

The effect of testosterone and methyl testosterone on guanidoacetic acid in blood and urine

- 9 John J Schneider (by invitation) and Harold L Mason, *Div of Biochemistry, Mayo Clinic, Rochester*

Substances isolated following the incubation of dehydroisoandrosterone, androsterone and etiocholanolone with liver tissue

- 10 Max L Sweat (by invitation) and L T Samuels, *Dept of Biochemistry, University of Utah School of Medicine, Salt Lake City*

An enzyme requiring DPN involved in the metabolism of testosterone by liver tissue

- 11 Louis Levin and Joseph W Jailer (by invitation), *Depts of Anatomy and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York*

The effect of gonadotrophic stimulation on the cholesterol content of the immature rat ovary

- 12 G A Wills, Sybil Rampton (by invitation) and L I Pugsley, *Food and Drugs Div, Dept National Health and Welfare, Ottawa, Canada*

Variables affecting the assay of testosterone propionate using male castrate rats

- 13 Lewis L Engel, Helen R Patterson and Hildeward Wilson (introduced by A Baird Hastings), *Medical Labs of the Collis P Huntington Memorial Hospital of Harvard University, at the Massachusetts General Hospital, Boston, and the Dept of Biological Chemistry, Harvard University Medical School, Boston*

The quantitative estimation of steroid alcohols

### BIOCHEMISTRY

Wednesday, March 17, 1 45 p m

ROOM C, CONVENTION HALL

Symposium on Methods for the Determination of the Purity of Substances of Biochemical Interest

VINCENT DU VIGNEAUD, *Chairman*

- 1 Dr George H Cassidy, of Yale University  
Chromatography
- 2 R Bowling Barnes, American Cyanamid Company, Stamford, Conn  
Spectrophotometry
- 3 Lyman C Craig, The Rockefeller Inst for Medical Research, New York  
Counter current distribution
- 4 J W Williams, University of Wisconsin, Madison  
Electrophoresis and ultracentrifugation
- 5 R M Herriott, The Rockefeller Inst for Medical Research, Princeton  
Solubility analysis
- 6 James Hillier, RCA Labs, Princeton  
Electron microscopy

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### Federation "Mixer"

Wednesday, March 17, 9 00 p m

ARENA, CONVENTION HALL

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### BIOCHEMISTRY A

Thursday, March 18, 9 00 a m

ROOM C, CONVENTION HALL

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### Carbohydrate Metabolism

- 1 Earl W Sutherland and Christian deDuve (introduced by Carl F Cori), Dept of Biochemistry, Washington University School of Medicine, St Louis  
A glycogenolytic factor from pancreas
- 2 George L Collins, Jr (by invitation), Edward M Bridge, and Barbara Matland (by invitation), Staller Research Labs of the Children's Hospital, Buffalo, and Dept of Pediatrics of the University of Buffalo  
The action of insulin on glycogen reserves
- 3 Michael Somogyi, Lab of the Jewish Hospital of St Louis  
A paradoxical effect of insulin on glucose assimilation
- 4 C A Villee, F M Sinev, and A K Solomon (introduced by A Baird Hastings), Dept of Biological Chemistry and the Biophysical Lab, Harvard University Medical School, Boston  
*In vitro* utilization of glucose by rat diaphragm muscle
- 5 Sigmund Schwimmer, Enzyme Research Lab, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U S Dept of Agriculture, Albany, Calif

Purification of alpha-amylase from barley malt extracts

- 6 Victor A Najjar, Dept of Biological Chemistry, Washington University School of Medicine, St Louis  
The purification and crystallization of phosphoglucomutase
- 7 Edwin G Krebs (by invitation) and Victor A Najjar, Dept of Biological Chemistry, Washington University School of Medicine, St Louis  
Immunochemical studies on purified d glyceraldehyde 3 phosphate dehydrogenase from yeast and rabbit muscle
- 8 Sidney F Velick, Dept of Biological Chemistry, Washington University School of Medicine, St Louis  
An electrophoretic analysis of the interaction of aldolase and glyceraldehyde phosphate dehydrogenase with phosphate ions
- 9 Karl Meyer and Charles Ragan (by invitation), Depts of Ophthalmology and Medicine, School of Medicine, Columbia University, New York  
Inhibition of hyaluronidase by hydroquinones and quinones
- 10 William H Fishman, Kurt I Altman (by invitation), and Belle Springer (by invitation), Depts of Surgery and Biochemistry, University of Chicago  
Blood plasma anti glucuronidase activity

### BIOCHEMISTRY B

Thursday, March 18, 9 00 a m

ROOM D, CONVENTION HALL

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### Amino Acid Metabolism (continued)

- 1 Theodore Winnick, Felix Friedberg (by invitation), and David M Greenberg, Div of Biochemistry, University of California Medical School, Berkeley  
Incorporation of C<sup>14</sup> labeled glycine into the protein of tissue homogenates
- 2 Henry Borsook, Clara L Deasy (by invitation), Jacob W Dubnoff, C T O Fong (by invitation), William D Fraser (by invitation), A J Haagen-Smit (by invitation), Geoffrey Keighley (by invitation), Peter H Lowry (by invitation), William G Kerckhoff Labs of the Biological Sciences, California Inst of Technology, Pasadena  
Protein and peptide turnover with respect to lysine in guinea pig liver homogenate
- 3 Philip P Cohen and Santiago Grisolia (by invitation), Dept of Physiological Chemistry, University of Wisconsin Medical School, Madison  
The mechanism of enzymatic synthesis of citrulline from ornithine

- 4 Ernest Borek and Heinrich Waelsch, *Depts of Biochemistry, New York State Psychiatric Inst and Columbia University, New York*  
The role of bicarbonate in glutamic acid metabolism
- 5 Heinrich Waelsch and Phyllis Owades (by invitation), *Depts of Biochemistry, New York State Psychiatric Inst and Columbia University, New York*  
The inhibition of glutaminase by glutamic acid
- 6 Alton Meister (by invitation) and Jesse P Greenstein, *National Cancer Inst, National Inst of Health, Bethesda, Md*  
Enzymatic hydrolysis of acetopyruvic acid
- 7 Vincent E Price (by invitation) and Jesse P Greenstein, *National Cancer Inst, National Inst of Health, Bethesda, Md*  
N acylated and N methylated glycyldehydroalanine and related compounds
- 8 David B Sprinson (by invitation) and D Rittenberg, *Dept of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*  
The metabolism of the  $\alpha$ ,  $\beta$  and  $\gamma$  hydrogen atoms of L leucine
- 9 Aaron Bunsen Lerner (by invitation), Thomas B Fitzpatrick (by invitation), Evan Calkins (by invitation), and William H Summerson, *Biochemistry Section, Medical Div, Army Chemical Center, Md*  
Enzymatic oxidation of tyrosine and dihydroxyphenylalanine by melanoma extracts

## BIOCHEMISTRY C

Thursday, March 18, 9 00 a m

ROOM B, CONVENTION HALL

### Pteroylglutamic Acid

- 1 Arnold D Welch, Robert W Heinle (by invitation), Jack A Pritchard (by invitation), and Herbert Salis, (by invitation), *Depts of Pharmacology and Medicine, Western Reserve University School of Medicine, Cleveland*  
Influence of pteroylglutamic acid on the synthesis and action of the antipernicious anemia factor (Nutrition)
- 2 R C Grubbs (by invitation), B C Houghton (by invitation), Julia Trossbach (by invitation), and F A Hitchcock, *The Lab of Aviation Physiology, Ohio State University, Columbus*  
The effects of folic acid on respiratory metabolism (Nutrition)
- 3 Paul L Day, Dorothy S Gaines (by invitation),

Marion McKee (by invitation), Phyllis Scroggin (by invitation), and Raymond Houchins (by invitation), *Dept of Biochemistry, School of Medicine, University of Arkansas, Little Rock*

Pteroylglutamic acid balance studies on monkeys (Nutrition)

- 4 Conrado F Asenjo, *Dept of Chemistry and Nutrition, School of Tropical Medicine, San Juan, Puerto Rico*

Folic acid requirement of the rat and some characteristic lesions observed in the deficient animals

- 5 Charles W Mushett (by invitation) and Gladys A Emerson, *Merck Inst for Therapeutic Research, Rahway, N J*

Response of leukopenia and granulocytopenia in sulfathiazole fed rats to pteroylglutamic acid and to parenteral liver extracts (Nutrition)

- 6 Gladys A Emerson and Charles W Mushett (by invitation), *Merck Inst for Therapeutic Research, Rahway, N J*

Influence of liver extracts on a sulfathiazole induced dietary deficiency in rats (Nutrition)

- 7 B S Schweigert (introduced by P B Pearson), *Dept of Biochemistry and Nutrition, A and M College of Texas, College Station*  
Studies on folic acid conjugase in blood

- 8 M E Swendseid (by invitation), E L Wittle (by invitation), G W Moersch (by invitation), O D Bird, and R A Brown, *The Research Labs, Parke, Davis and Co, Detroit*

Studies in the rat of inhibitors of pteroylglutamic acid structurally related to this vitamin (Nutrition)

- 9 E L R Stokstad, M Regan (by invitation), A L Franklin (by invitation), and T H Jukes, *Lederle Labs Div, American Cyanamid Co, Pearl River, N Y*

Interrelationships between pteroylglutamic acid, adenine thymine, and antagonists of pteroylglutamic acid

- 10 George H Hitchings, Gertrude B Elion (by invitation), and Henry VanderWerff (by invitation), *The Wellcome Research Labs, Tuckahoe, N Y*

a-Aminopurine as a purine antagonist

- 11 John R Totter and Edith S Sims (by invitation), *Dept of Biochemistry, School of Medicine, University of Arkansas, Little Rock*

The influence of KCN and pteroylglutamic acid on growth and porphyrin production of *Corynebacterium hoffmannii*

**BIOCHEMISTRY D**

Thursday, March 18, 9 00 a m

ROOM S, CONVENTION HALL

**Sulfur Compounds**

- 1 **J P Saunders and W A Himwich** (introduced by Bernard J Jandorf), *Toxicology Section, Medical Div, Army Chemical Center, Md*  
Some properties of a transsulfurase responsible for conversion of cyanide to thiocyanate
- 2 **Otto Rosenthal**, *Harrison Dept of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia*  
The distribution of rhodanese
- 3 **C Boyd Shaffer and Frances H Critchfield** (introduced by Leonard H Cretcher), *Chemical Hygiene Fellowship, Mellon Inst, Pittsburgh*  
Lipotropic activity and toxicity of methionine (oxy-methionine)
- 4 **Herbert C Tidwell** (introduced by Max N Huffman), *Dept of Biochemistry, Southwestern Medical College, Dallas*  
Some factors which influence methionine excretion in the rat
- 5 **Cosmo G Mackenzie, Julian R Rachele** (by invitation), *Nancy Cross* (by invitation), *Joseph P Chandler, and Vincent duVigneaud*, *Dept of Biochemistry, Cornell University Medical College, New York*  
Study of the oxidation of the labile methyl group of dietary methionine traced with  $C^{14}$
- 6 **Jacob W Dubnoff and Henry Borsook**, *William G Karchhoff Labs of the Biological Sciences, California Inst of Technology, Pasadena*  
Dimethylthetin as a methylator of homocysteine
- 7 **Jakob A Stekol**, *The Lankenau Hospital Research Inst, and the Inst for Cancer Research, Philadelphia*  
Synthesis of S-benzyl thiopyruvic acid and its conversion to N acetyl S-benzyl cysteine in the rat
- 8 **Kay Nakamura** (by invitation) and **Francis Binkley**, *Lab for the Study of Hereditary and Metabolic Disorders and the Depts of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City*  
The enzymatic hydrolysis of glutathione
- 9 **Julius Schultz** (introduced by Harry M Vais), *Harrison Dept of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia*  
Influence of the presence of a sterile abscess on the detoxication of brombenzene as mercapturic acid

**BIOCHEMISTRY A**

Thursday, March 18, 1 15 p m

ROOM C, CONVENTION HALL

**Symposium on Hemin Pigments and Chromoproteins**David L Drabkin, *Chairman*

- 1 **David L Drabkin**, *University of Pennsylvania, Philadelphia*  
Distribution and metabolic aspects of derivatives of iron protoporphyrin (hemin)
- 2 **Paul F Hahn**, *Meharry Medical College, Nashville*  
Metabolism of iron
- 3 **W Mansfield Clark**, *Johns Hopkins University, Baltimore*  
A systematic treatment of coordination complexes of iron protoporphyrins with nitrogenous bases
- 4 **Jeffries Wyman, Jr**, *Harvard University, Cambridge*  
The relation of physiological function and molecular structure in hemoglobin
- 5 **Leonor Michaels**, *The Rockefeller Inst for Medical Research, New York*  
Molecular oxygen as a ligand in metal porphyrins and other metal complex compounds

**BIOCHEMISTRY BUSINESS MEETING**

Thursday, March 18, 4 15 p m

ROOM C, CONVENTION HALL

**BIOCHEMISTRY B**

Thursday, March 18, 1 45 p m

ROOM D, CONVENTION HALL

**Glycolysis**

- 1 **Chalmers L Gemmill**, *Dept of Pharmacology, University of Virginia Medical School, Charlottesville*  
Inhibitory effects of naphthoquinones and related compounds on glycolysis (Pharmacol)
- 2 **Helena Gilder** (introduced by Vincent duVigneaud), *Marion H Wilson* (by invitation), and *Johanna M Lee* (by invitation), *Dept of Biochemistry, Cornell University Medical College, New York*  
Studies on glycolysis of mouse lymphosarcoma
- 3 **William H Summerson**, *Helena Gilder* (by invitation), and *Johanna M Lee* (by invitation), *Dept of Biochemistry, Cornell University Medical College, New York*  
Effect of pH on the aerobic metabolism of lymphosarcoma cells

- 4 Robert M Bird and John D Evans (introduced by William H Summerson), *Dept of Physiology, Cornell University Medical College, New York*

Effect of pH on the aerobic metabolism of rabbit bone marrow

- 5 Marion K Birmingham (by invitation) and K A C Elliott, *Montreal Neurological Inst, McGill University, Canada*

Effects of pH and bicarbonate on brain tissue respiration and anaerobic glycolysis

- 6 K A C Elliott, *Montreal Neurological Inst, McGill University, Canada*

Metabolism of cerebral cortex from different areas and various animals and from epileptic patients

- 7 James A Bain (by invitation) and J Raymond Klein, *Depts of Pharmacology, Psychiatry, and Biological Chemistry, University of Illinois College of Medicine, Illinois Neuropsychiatric Inst, Chicago*

Effect of carbon dioxide concentration on brain lactate

- 8 J Raymond Klein and James A Bain (by invitation), *Depts of Psychiatry, Biological Chemistry, and Pharmacology, University of Illinois College of Medicine, Illinois Neuropsychiatric Inst, Chicago*

Effect of carbon dioxide concentration on the changes in brain metabolites accompanying convulsions

- 9 Winifred Ashby (introduced by Joseph H Roe), *Blackburn Lab, St Elizabeths Hospital, Washington*

Distribution pattern of carbonic anhydrase in the fetal central nervous system

## BIOCHEMISTRY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM C, CONVENTION HALL

### BIOCHEMISTRY C

Thursday, March 18, 1 45 p m

ROOM B, CONVENTION HALL

#### Nutrition

- 1 Robert A Harte (by invitation), John J Travers (by invitation), Peter Sarich (by invitation), and James B Allison, *Research Labs, Arlington Chemical Co, Yonkers*

The effect of protein quality of previous intake on the consequences of acute starvation

- 2 James B Allison, John A Anderson (by invitation), and John I White (by invitation), *Bureau of Biological Research, Rutgers University, New Brunswick*

Repletion of protein depleted dogs with whole egg and wheat gluten proteins

- 3 Bacon F Chow and Shirley DeBiase (by invitation), *Div of Protein Chemistry, The Squibb Inst for Medical Research, New Brunswick*

Repletion of protein depleted dogs with various proteins and protein hydrolysates

- 4 Charles F Kade, Jr and Jesse Shepherd (introduced by Aaron Arnold), *Biology Div, Sterling Winthrop Research Inst, Rensselaer, N Y*

The inhibitory effect of excess methionine on protein utilization (Nutrition)

- 5 Barnett Sure and Freeland Romans (by invitation), *University of Arkansas, Fayetteville*

Influence of the concentration of the vitamin B complex on protein utilization

- 6 Oliver H Gaebler and Paul Bartlett (by invitation), *Henry Ford Hospital, Detroit*

Conversion of protein to glucose in vitamin deficiencies

- 7 Anthony A Albanese, L E Holt, Jr, and Virginia I Davis (by invitation), Selma E Snyderman (by invitation), Marilyn Lein (by invitation), and Emilie M Smetak (by invitation), *New York University College of Medicine*

The sulfur amino acid requirement of the infant

- 8 Wendell H Griffith and Mary F Nawrocki (by invitation), *Dept of Biological Chemistry, St Louis University School of Medicine*

The effect of threonine in choline deficiency (Nutrition)

- 9 W W Westerfeld and J M McKibbin (by invitation), *Dept of Biochemistry, Syracuse University College of Medicine, Syracuse, New York*

Acetaldehyde metabolism in dogs maintained on a purified diet

## BIOCHEMISTRY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM C, CONVENTION HALL

### BIOCHEMISTRY A

Friday, March 19, 9 00 a m

ROOM C, CONVENTION HALL

#### Tricarboxylic Acid Cycle

- 1 Murray Saffran and J L Prado (introduced by K A C Elliott), *Dept of Biochemistry, McGill University, Montreal, Canada*

Oxidation of members of the Krebs cycle by liver and kidney Inhibition by *trans*-aconitate

- 2 **B L Horecker and J N Stannard** (by invitation), *Lab of Physical Biology, National Inst of Health, Bethesda, Md*

The kinetics of inhibitor action in a carrier-linked system

- [3 **D T Watts** (introduced by C L Gemmell), *Dept of Pharmacology, University of Virginia Medical School, Charlottesville*

Effect of some central nervous system stimulants and depressants on the activity of succinic dehydrogenase (Pharmacol)

- 4 **J Leyden Webb** (by invitation), **Paul R Saunders** (by invitation), and **Clinton H Thienes**, *Dept of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles*

Metabolism of rat heart slices (Pharmacol)

- 5 **O N Miller** (by invitation), **R E Olson** (by invitation), and **F J Stare**, *Dept of Nutrition, Harvard University School of Public Health, and Dept of Biological Chemistry, Harvard University Medical School, Boston*

Utilization of pyruvate by heart ventricle *in vitro* as a function of time

- 6 **R E Olson** (by invitation), **O N Miller** (by invitation), and **F J Stare**, *Dept of Nutrition, Harvard University School of Public Health, and Dept of Biological Chemistry, Harvard University Medical School, Boston*

Effect of biotin deficiency upon the respiration of cardiac muscle in ducklings

- 7 **J Ciethaml** (by invitation), **M C Gollub** (by invitation), **J Speck** (by invitation), and **B Vennesland**, *Dept of Biochemistry, University of Chicago*

Some properties of  $\beta$ -keto acid carboxylases from plants

- 8 **F Edmund Hunter, Jr**, *Dept of Pharmacology, Washington University School of Medicine, St Louis*

Phosphorylation of glucose due to a coupled oxidation-reduction between  $\alpha$ -ketoglutaric acid and oxalacetic acid

- 9 **Morris E Friedkin** (by invitation) and **Albert L Lehninger**, *Depts of Biochemistry and Surgery, University of Chicago*

Oxidation-coupled phosphate exchanges in the acid-insoluble esters of cell-free liver preparations

- 10 **George Kalnitsky** (introduced by H A Mattill), *Dept of Biochemistry, State University of Iowa, Iowa City*

Citrate formation from oxalacetate

- 11 **Samuel Natelson, Joseph B Pincus, and Julius K Lugovoy** (introduced by Albert E Sobel), *Dept of Biochemistry of the Jewish Hospital of Brooklyn*

Response of citric acid levels to administration of glucose

## BIOCHEMISTRY B

Friday, March 19, 9 00 a m

Room D, CONVENTION HALL

### Proteolytic Enzymes

- 1 **Emil L Smith**, *Lab for the Study of Hereditary and Metabolic Disorders, and the Depts of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City*  
The hydrolysis of glycylglycine and glycyl-L leucine by peptidases
- 2 **H Theo Hanson** (by invitation) and **Emil L Smith**, *Lab for the Study of Hereditary and Metabolic Disorders, and the Depts of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City*  
Action of peptidases on  $\beta$  alanine peptides
- 3 **C E Carter** (introduced by J P Greenstein), *Clinton National Lab, Oak Ridge, Tenn*  
The metabolism of peptides of asparagine
- 4 **Hans Neurath and Elaine Elkins** (by invitation), *Dept of Biochemistry, Duke University School of Medicine, Durham*  
Kinetics and inhibition of carboxypeptidase activity
- 5 **Helmut R Gutmann** (by invitation) and **Joseph S Fruton**, *Dept of Physiological Chemistry, Yale Univ, New Haven*  
Partial purification of cathepsin II from swine kidney
- 6 **Elizabeth K Patterson, Marjorie E Dackerman, and Jack Schultz** (introduced by Theodore F Lavine), *Lankenau Hospital Research Inst and Inst for Cancer Research, Philadelphia*  
Peptidase increase during growth of the salivary glands of the fly, *Drosophila melanogaster*
- 7 **Otto Schales, Regina M Roux** (by invitation), and **Anne M Suthon** (by invitation), *Chemical Research Lab of the Alton Ochsner Medical Foundation, and Dept of Biochemistry, Tulane University, New Orleans*  
Inhibition of peptic activity by hydrazine
- 8 **Heinz Fraenkel-Conrat, R S Bean** (by invitation), and **Hans Lineweaver**, *Western Regional Research Lab, Albany, Calif, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U S Dept of Agriculture*  
Concerning the mechanism of interaction of egg white trypsin inhibitor and trypsin
- 9 **Donald E Bowman**, *Dept of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis*  
Inactivation of trypsin by acetylation and iodination

## BIOCHEMISTRY C

Friday, March 19, 9 00 a m  
ROOM B, CONVENTION HALL

## Antibiotics and Bacterial Metabolism

- 1 Ibert C Wells (by invitation), William H Elliott (by invitation), Sidney A Thayer, and Edward A Doisy, *Lab of Biological Chemistry, St Louis University School of Medicine*  
Ozonolysis of the Pyo compounds
- 2 M Adler (by invitation) and O Wintersteiner, *Div of Organic Chemistry, The Squibb Inst for Medical Research, New Brunswick*  
The penicillins produced by *Aspergillus flavus*
- 3 James D Dutcher, O Wintersteiner, and A E O Menzel (by invitation), *Div of Organic Chemistry, The Squibb Inst for Medical Research, New Brunswick*  
Structural investigation of hydroxyaspergillie acid, an antibiotic substance produced by *Aspergillus flavus*
- 4 Kent Wight (by invitation) and Dean Burk, *National Cancer Inst, National Inst of Health, Bethesda, Md*  
The effect of streptomycin on deamination and oxygen consumption by resting cells of *E coli*
- 5 Ralph W McKee and Quentin M Geiman (by invitation), *Depts of Biological Chemistry and of Comparative Pathology and Tropical Medicine, Harvard University Medical School, Boston*  
Methionine in the growth of the malarial parasite, *Plasmodium knowlesi*
- 6 G M Shull (by invitation), Richard W Thomas (by invitation), and W H Peterson, *Dept of Biochemistry, University of Wisconsin, Madison*  
Nature of the "sporogenes vitamin" and nutrition of *Clostridium sporogenes*
- 7 Gerrit Toennies and Dorothy L Gallant (by invitation), *Lankenau Hospital Research Inst and Inst for Cancer Research, Philadelphia*  
On the bacterial metabolism of lysine
- 8 Carl M Lyman and K A Kuiken (by invitation), *Dept of Biochemistry and Nutrition, A and M College of Texas, College Station*  
Effect of vitamin B<sub>6</sub> on the utilization of D amino acids by lactic acid bacteria
- 9 Muriel M Burr and W A Crandall (introduced by L I Pugsley), *Food and Drugs Div, Dept National Health and Welfare, Ottawa, Canada*  
A study of the microbiological assay of valine with estimation of precision of the method

## BIOCHEMISTRY D

Friday, March 19, 9 00 a m  
ROOM S, CONVENTION HALL

## Thyroid and Miscellaneous Papers

- 1 Marschelle H Power, William C McConahey, Jr (by invitation), F Raymond Keating, Jr (by invitation), and Joseph Berkson (by invitation), *Divs of Biochemistry, Medicine, and Biometry and Medical Statistics, Mayo Clinic, Rochester*  
Preliminary observations on the renal excretion of radioiodine after administration of tracer doses
- 2 DeWitt Stetten, Jr, and Adele Karp (by invitation), *Dept of Biochemistry, College of Physicians and Surgeons, Columbia University, New York*  
Influence of the thyroid incorporation of deuterium into tissue constituents of the rat
- 3 David L Drabkin, *Dept of Physiological Chemistry, Graduate School of Medicine, University of Pennsylvania, Philadelphia*  
The effect of thyroidectomy and of thiouracil on cytochrome C metabolism and liver regeneration
- 4 Richard J Winzler and Earl Frieden (by invitation), *Dept of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles*  
Thyroxine activity and antagonism of some structural analogues of thyroxine
- 5 J F McClendon, William C Foster (by invitation), and J W Cavett (by invitation), *Hahnemann Medical College, Philadelphia, and Salsbury's Labs, Charles City, Iowa*  
Pituitary protein-bound iodine and the Plummer treatment of hyperthyroidism
- 6 Leonard T Skeggs (by invitation), Jack R Leonards (by invitation), and Victor C Myers, *Dept of Clinical Biochemistry, Western Reserve University, Cleveland*  
The effect of hypertrophy on the chemical composition of rat cardiac muscle
- 7 Roger W Marsters (by invitation), Jack R Leonards (by invitation), and Victor C Myers, *Dept of Clinical Biochemistry, Western Reserve University, Cleveland*  
The chemical composition of human aortas
- 8 Eugene Roberts (introduced by Michael Somoogy), *Barnard Free Skin and Cancer Hospital, St Louis*  
Determination of arginase activity in tissue homogenates application to epidermal carcinogenesis in mice
- 9 F B Seibert, M L Pfaff (by invitation), and

M V Seibert (*by invitation*), Henry Phipps Inst, University of Pennsylvania, Philadelphia

A quantitative method for a serum polysaccharide present in cases of tuberculosis and carcinoma

## BIOCHEMISTRY

### Papers Read by Title

- 1 Major Roberto Acosta, Mexican Army (*by invitation*) and Robert E Johnson, U S Army Medical Nutrition Lab, Chicago

A micromodification of the Folin method for estimating urinary creatinine

- 2 James C Andrews, Dept of Biological Chemistry and Nutrition, School of Medicine, University of North Carolina, Chapel Hill

Partial hydrolysis products of human hair

- 3 Reginald M Archibald and E Stroh (*by invitation*), Dept of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore

Fractionation of urinary steroids by use of the Craig counter current distribution machine

- 4 Howard H Beard, Dept of Biochemistry, Chicago Medical School

Correlation of the unitarian or trophoblastic thesis with the biological test of malignancy

- 5 Maurice Bruger, Samuel Member (*by invitation*), and Estelle Goldman (*by invitation*), Medical Research Lab, Dept of Medicine, New York Post Graduate Medical School and Hospital

The glucose content of the rat carcass

- 6 R L Dryer (*by invitation*), J Katz (*by invitation*), W D Paul (*by invitation*), and J I Routh, Depts of Biochemistry and Internal Medicine, College of Medicine, State University of Iowa, Iowa City

The anti ulcer activity of aluminum dihydroxy aminoacetate

- 7 John D Ferry and Sidney Shulman (*by invitation*), Dept of Chemistry, University of Wisconsin, Madison

Influence of polyhydric alcohols on the clotting of fibrinogen

- 8 D Breese Jones, Alvin Caldwell (*by invitation*), and Millard J Horn (*by invitation*), Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U S Dept of Agriculture, Washington

Availability of DL-lanthionine for the promotion of growth when added to a cystine-deficient diet

- 9 Harlan L Klug (*by invitation*), George P Lampson (*by invitation*), and Alvin L Moxon, Chemistry Dept, South Dakota

Agricultural Experiment Station, South Dakota State College, Brookings

Selenium tetraglutathione

- 10 Victor E Levine and Sidney Merlis (*by invitation*), Dept of Biological Chemistry and Nutrition, Creighton University School of Medicine, Omaha

Dibenzothiophene is a reagent for aldehydes, ketones and carbohydrates

- 11 Maurice V L'Heureux (*by invitation*), Wilbur R Tweedy, and Elinor M Zorn (*by invitation*), Dept of Biological Chemistry, Loyola University School of Medicine, Chicago

The excretion of labeled calcium by normal and thyroparathyroidectomized rats

- 12 George H Mangun, Dept of Labs, Henry Ford Hospital, Detroit

Effect of cytochrome C on the resistance of mice to anoxia

- 13 Mary E Mayer and Antoinette Greco (*by invitation*), National Cancer Inst, Bethesda, Md

The hydrolysis of nuclear proteins by cathepsins I Calf thymus cathepsin

- 14 Dale K Mechem (*by invitation*) and Harold S Olcott, Western Regional Research Lab, Albany, Calif, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U S Dept of Agriculture

An egg yolk protein containing 10% phosphorus

- 15 Samuel Natelson (*by invitation*), Benjamin Kramer, and Marvin Sherman (*by invitation*), Pediatric Research Lab, Jewish Hospital of Brooklyn

Blood sugar changes following the administration of lactose in raw and evaporated milk

- 16 Joseph M Quashnock (*introduced by* William S McElroy), Dept of Physiological Chemistry, School of Medicine, University of Pittsburgh

Gas pressure regulation in flame photometry

- 17 G G Rudolph (*by invitation*) and L T Samuels, Dept of Biochemistry, University of Utah School of Medicine, Salt Lake City

Early changes in the seminal vesicles of the castrate rat following administration of testosterone

- 18 Max Schlamowitz (*by invitation*) and David M Greenberg, Div of Biochemistry, University of California Medical School, Berkeley

Purification of phosphoglucomutase with "carbitol" acetate

- 19 Mona Spiegel-Adolf and Arnold S J Lee (*by invitation*), Dept of Colloid Chemistry, Temple University School of Medicine, Philadelphia

Polarographic studies in cerebrospinal fluids

- 20 Mario Stefanini (*introduced by* A J Quick),



*Dept of Internal Medicine, University of Roma, Italy, and Dept of Biochemistry, Marquette University School of Medicine, Milwaukee*

Mechanism of hyperbilirubinemia due to sodium nicotinate

- 21 **E L R Stokstad, J Pierce** (*by invitation*), **T H Jukes**, and **A L Franklin** (*by invitation*), *Lederle Labs Division, American Cyanamid Co, Pearl River, N Y*

The inhibition of pteroylglutamic acid conjugase by glutamic acid peptides of p-aminobenzoic acid

- 22 **M K Walden** (*introduced by A K Balls*), *Enzyme Research Lab, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U S Dept of Agriculture, Albany, Calif*  
Serine content of purothionin

## AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC

### THIRTY-EIGHTH ANNUAL MEETING

#### PHARMACOLOGY A

*Tuesday, March 16, 9 00 a m*

ROOM E, CONVENTION HALL

#### Sympathetic and Related Agents

- 1 **Carlton C Hunt** (*introduced by McKeen Cuttell*), *Department of Pharmacology, Cornell University Medical College and Sloan Kettering Institute, New York*

Structural relationship to sympatholytic activity of certain  $\beta$  chloroethyl amines

- 2 **Raymond P Ahlquist**, *Department of Pharmacology, University of Georgia School of Medicine, Augusta*

Comparative effects of sympathomimetic amines on the vasomotor resistance of the kidney, mesentery and leg

- 3 **Fred Shaffer** (*by invitation*), **P L Ewing** and **G A Emerson**, *Department of Pharmacology, University of Texas Medical Branch, Galveston*

Comparative effects of d desoxyephedrine and the isomers of amphetamine on smooth muscle

- 4 **Solomon Garb** (*by invitation*) and **Maynard B Chenoweth**, *Dept of Pharmacology, Cornell Univ Medical College, New York*  
Studies on ventricular fibrillation produced by epinephrine during hydrocarbon inhalation

- 5 **James A Richardson** (*by invitation*) and **R P Walton**, *Dept of Pharmacology, Medical College of South Carolina*

Further analysis of the influence of autonomic innervation on drug responses of the heart and gut in unanesthetized dogs

- 6 **Theodore O King**, *Department of Pharmacology, Georgetown University School of Medicine, Washington*

The effect of pitressin on the hemodynamic responses of epinephrine and N isopropyl-nor epinephrine

- 7 **William A Wolff** and **Marina Hawkins** (*by invitation*), *Tobacco Research Laboratory, Bowman Gray School of Medicine, Wake Forest College, Winston Salem*

A spectrophotometric method for nicotine in blood (Biochem)

- 8 **Amedeo S Marrazzi**, *Department of Pharmacology and Therapeutics, Wayne University College of Medicine, Detroit*  
Antagonism of nicotine and atropine

- 9 **John B Stanbury** (*introduced by Otto Kraye*), *Department of Pharmacology, Harvard Medical School*

The blocking action of magnesium ion on sympathetic ganglia

- 10 **Clem A Stone** (*by invitation*), **Paul Achenbach** (*by invitation*) and **Earl R Loew**, *Dept of Pharmacology, University of Illinois College of Medicine, Chicago*

Adrenergic blocking properties of certain halogenated ethylamine derivatives

- 11 **J A Wells** and **David P Rall** (*by invitation*), *Department of Pharmacology, Northwestern University Medical School, Chicago*

The influence of N-(2 bromoethyl) N ethyl-1 naphthalene methylamine on the vasopressor response of a series of amines

- 12 **B Richards** (*by invitation*), **A Cameron** (*by invitation*), **B Craver**, **E Herrold** (*by invitation*), *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products Inc, Summit, N J*

Detoxification of 2-benzyl-imidazolone hydrochloride (Piscol) by incubation with hepatic slices from the rat

### PHARMACOLOGY B

Tuesday, March 16, 9 00 a m

ROOM 9, CONVENTION HALL

#### General and Local Anesthetics

- 1 Duncan E Hutcheon (introduced by G H W Lucas), Department of Pharmacology, University of Toronto

Sudden deaths during chloroform and cyclopropane anaesthesia

- 2 Barbara Rennick (by invitation), S Donald Malton (by invitation), and Gordon K Moe, Department of Pharmacology, the University of Michigan, Ann Arbor

The effect of cyclopropane on the work capacity of the dog heart

- 3 Charles H Burnett (by invitation), Esther B Gordon (by invitation), Gerald Shortz (by invitation), David W Compton (by invitation) and Henry K Beecher, Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital and the Department of Medicine, Massachusetts General Hospital

A comparison of the effects of ether and cyclopropane on renal function in man

- 4 John C Krantz, Jr and C Jelleff Carr, Department of Pharmacology, University of Maryland, School of Medicine, Baltimore

Anesthesia with cyclobutane

- 5 C Jelleff Carr and John C Krantz, Jr, Department of Pharmacology, University of Maryland, School of Medicine, Baltimore

A comparative study of cyclic and noncyclic hydrocarbons on cardiac automaticity

- 6 H R Hulpieu and V V Cole, Department of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis

Procaine metabolism in dogs anesthetized by thiopental, ether and chloroform

- 7 Philip A Lief (by invitation), Raymond Poet (by invitation) and Bernard B Brodie, Departments of Anesthesiology and Biochemistry, New York University College of Medicine, and New York University Research Service, Goldwater Memorial Hospital, New York

The physiological disposition of procaine in man

- 8 Alexander G Karczmar (by invitation) and Theodore Koppanyi, Department of Pharma-

cology and Materia Medica, Georgetown University School of Medicine, Washington

Action of central nervous system depressants at different growth periods of salamander (*Amblystoma punctatum*) larvae

- 9 Theodore Koppanyi and Alexander G Karczmar (by invitation), Dept of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington

Comparison of anesthetic action of acetanilid, trichloro (MS 222) and aliphatic depressants

### PHARMACOLOGY C

Tuesday, March 16, 9 00 a m

ROOM 1, CONVENTION HALL

#### Drug Toxicity

- 1 R A Gardner (by invitation), P L Ewing, G A Emerson and A E Hansen, Departments of Pediatrics and Pharmacology, University of Texas Medical Branch, Galveston

Synergism in relation to toxicity of certain antiasthmatic drugs

- 2 J K Finnegan, P S Larson and H B Haag, Department of Pharmacology, Medical College of Virginia, Richmond

Comparative tolerance of dogs, cats and rabbits to nicotine

- 3 David I Macht and Marcus Ostro (by invitation), Departments of Pharmacology and Radiology, Sinai Hospital, Baltimore

Phytotoxic effects of normal, pathological, and irradiated blood sera

- 4 Ruth Musser (introduced by John C Krantz, Jr), Department of Pharmacology, Univ of Maryland, School of Medicine, Baltimore

Studies with the polyoxyethylene derivatives of sorbitan partial esters (Tweens)

- 5 Alfred G Lisi (introduced by Charles M Gruber), Department of Pharmacology, Jefferson Medical College, Philadelphia

The toxicity of some surface active agents on the frog heart

- 6 Charles H Hine and Herbert E Christensen (by invitation), Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco

The comparative activity of some  $\alpha$  substituted glycerol and glycidyl ethers

- 7 Norman W Karr and Edward L Hendricks (introduced by Norman A David), Department of Pharmacology, University of Oregon Medical School, Portland

Toxicity of intravenous ammonium salts

- 8 A C Conway (by invitation), F S Ting (by invitation), and Julius M Coon, Department of Pharmacology, University of Chicago

Effect of anti cholinesterases upon procaine toxicity

- 9 O Garth Fitzhugh, Arthur A Nelson and Oma L Holland (*by invitation*), *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
The chronic toxicity of thiourea

- 10 Paul K Smith, Abraham I Gimble (*by invitation*) and Clarke Davison (*by invitation*), *Department of Pharmacology, The George Washington University School of Medicine, Washington*  
The tissue distribution and toxicity of emetine

- 11 Frieda M Kunze (*by invitation*) and Edwin P Laug, *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
The penetration of lead through the skin of the rat

- 12 Frederick G Germuth, Jr (*by invitation*) and Harry Eagle, *Laboratory of Experimental Therapeutics of the U S Public Health Service and the Johns Hopkins School of Hygiene and Public Health, Baltimore*  
The efficacy of BAL (2,3 Dimercaptopropanol) in the treatment of experimental lead poisoning in rabbits

- 13 Lehman M Lusk (*by invitation*), Herbert A Braun and Edwin P Laug, *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
The protective action of BAL on experimental poisoning by lead, tungsten, copper and Paris green

### Symposium on Human Pharmacological Experiments

- History and Ethics of Human Physiological and Pharmacological Experiments  
*Speaker, A C Ivy*  
*Discussant, C F Schmidt*
- Autonomic Drugs Adaptable to Human Experimentation  
*Speaker, I Starr*  
*Discussant, J Comroe, Jr*
- Analgesic and Anesthetic Drugs in Man  
*Speaker, E G Gross*  
*Discussant, G L Maison*
- Miscellaneous Human Pharmacological Experiments  
*Speaker, K Unna*  
*Discussant, H Bruner*
- Dangers, Safeguards and Necessary Precautions  
General Discussion

### PHARMACOLOGY A

Wednesday, March 17, 9 00 a m

ROOM E, CONVENTION HALL

#### Analgesics

- Robert A Lehman, Herbert S Kupperman (*by invitation*) and Joy Phillips (*by invitation*), *Department of Therapeutics, New York University College of Medicine*  
Studies on the relationship between chemical constitution and analgesic activity
- Carl C Pfeiffer, J Santos-Martinez (*by invitation*) and Theodore R Sherrod (*by invitation*), *Department of Pharmacology, University of Illinois College of Medicine, Chicago*  
The nature of the prosthetic groups of analgesics and their possible action as blocking agents
- Nilkanth M Phatak, James Maloney (*by invitation*) and Norman David, *Department of Pharmacology, University of Oregon Medical School, Portland*  
Use of hyperglycemic response for estimating addiction potentialities of analgesic compounds
- F P Luduena, Lloyd C Miller, Estelle Ananenko (*by invitation*) and J D Frick (*by invitation*), *Biology Division, Sterling Winthrop Research Institute, Rensselaer, N Y*  
Some pharmacological actions of isomers of methadon
- Elizabeth H Jenney (*by invitation*) and Carl C Pfeiffer, *Department of Pharmacology, University of Illinois College of Medicine, Chicago*

### JOINT SESSION OF THE FEDERATION

Tuesday, March 16, 1 30 p m

BALLROOM, CONVENTION HALL

Program on page 314

### PHARMACOLOGY BUSINESS MEETING

Tuesday, March 16, 4 15 p m

ROOM E, CONVENTION HALL

### PHARMACOLOGY

Tuesday, March 16, 7 30 p m

BALLROOM, CONVENTION HALL

Comparative analgesic and toxic effects of the optical isomers of methadon and isomethadon

- 6 **Theodore R Sherrod** (*by invitation*), **Robert Kaiser** (*by invitation*), **J Santos-Martinez** (*by invitation*) and **Carl C Pfeiffer**, *Department of Pharmacology, University of Illinois College of Medicine, Chicago*

Methadon derivatives of pharmacological interest

- 7 **James O Hoppe** (*by invitation*) and **Lloyd C Miller**, *Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N Y*

A comparison of the toxicity of methadon and some related compounds

- 8 **Jane E Denton** (*by invitation*), **Oliver H Straus** (*by invitation*), **William E Waddell** (*by invitation*) and **Henry K Beecher**, *Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital, Boston*

A comparison of side actions and analgesic effects of morphine, amudone (1:4 diphenyl 6 dimethylamino heptanone 3) and its isomers in man

- 9 **Robert C Batterman** and **Abraham M Oshlag** (*by invitation*), *Department of Therapeutics, New York University College of Medicine*

The effectiveness and toxicity of methadon

- 10 **Marion Bell** (*by invitation*) **Joan E Copeland** (*by invitation*) and **Eldon M Boyd**, *Dept of Pharmacology, Queen's Univ Kingston, Canada*

On the mechanism of the antitussive action of amudone

- 11 **G S Eadie**, **F Bernheim** and **D B Fitzgerald** (*by invitation*), *Dept of Physiology and Pharmacology, Duke University School of Medicine, Durham*

The effect of methadon on the cholinesterase of the brain

- 12 **Ted A Loomis** (*introduced by James M Dille*), *Department of Pharmacology, University of Washington School of Medicine, Seattle*

The response and the mechanism of the response of the duodenum to morphine

- 13 **Sydney Ellis**, *Department of Physiology and Pharmacology, Duke University School of Medicine, Durham*

Enzymic hydrolysis of morphine esters

- 14 **Harris Isbell** (*by invitation*) and **A J Eiseman**, *Research Department, U S Public Health Service Hospital, Lexington, Ky*

Physical dependence liability of drugs of the methadon series and of 6 methyl-dihydromorphine (Biochem)

## PHARMACOLOGY B

Wednesday, March 17, 9 00 a m

Room 9, CONVENTION HALL

### Antibiotics and Chemotherapeutic Agents

- 1 **R J Schachter** (*introduced by N Ercoli*), *Department of Pharmacology and Chemotherapy, Warner Institute of Therapeutic Research, New York*

The circulation of penicillin in the lymph

- 2 **N Ercoli**, **M N Lewis** (*by invitation*), **B S Schwartz** (*by invitation*), **M H Whitehead** (*by invitation*), *Dept of Pharmacology and Chemotherapy, Warner Institute of Therapeutic Research, New York*

The therapeutic significance of detectable penicillin levels

- 3 **Harold M Peck**, **Horace F Russo**, **Elizabeth K Tillson**, **William S Waller** (*all by invitation*) and **Karl H Beyer**, *Department of Pharmacology, The Medical Research Division, Sharp and Dohme, Inc, Glenolden, Pa*

The renal elimination of caronamide (4' carboxyphenyl methanesulfonamide)

- 4 **Hans Molitor** and **Samuel Kuna** (*by invitation*), *Merck Institute for Therapeutic Research, Rahway*

Pharmacologic studies of the neurotoxic properties of streptomycin

- 5 **Joseph E Hawkins Jr** and **Walter J O'Shanny** (*by invitation*), *Merck Institute for Therapeutic Research, Rahway*

Functional analysis of the chronic neurotoxic action of streptomycin

- 6 **Lloyd D Seager**, *Department of Pharmacology, The Woman's Medical College of Pennsylvania*

The chemotherapeutic action of a number of furan derivatives

- 7 **Andres Goth** and **Fabian J Robinson** (*by invitation*), *Department of Physiology and Pharmacology, Southwestern Medical College, Dallas*

Chemotherapeutic studies on a series of dithiocarbamates and their bismuth derivatives

- 8 **David M Tennent** and **Margaret L Leland** (*introduced by Randolph T Major*), *Merck Institute for Therapeutic Research, Rahway*

A procedure for the determination of 4 amino salicylic acid in blood and urine (Biochem)

- 9 **Les Loewe** (*by invitation*), **Albert Sobel**, **Oscar Gawron** (*by invitation*) and **Erna Altewerber** (*by invitation*), *Departments of Medicine and Biochemistry, Jewish Hospital of Brooklyn*

New penicillin products for prolonged blood levels (Biochem)

- 10 John V Scudi and Charles J Duca (by invitation), *Nepera Chemical Co, Inc, Yonkers*  
Bacteriological characteristics of mandelamine (Biochem)
- 11 R M Archibald and James R Weisiger (by invitation), *Hospital of The Rockefeller Institute for Medical Research, New York*  
Reaction of plasmochin with formaldehyde (Biochem)

### PHARMACOLOGY C

Wednesday, March 17, 9 00 a m

ROOM 1, CONVENTION HALL

#### Parasympathetic and Related Agents

- 1 Clara Torda and Harold G Wolff, *New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York*  
Effect of convulsant and anticonvulsant agents on acetylcholine metabolism and effector organs acetylcholine sensitivity
- 2 K R Unna, K Glaser (by invitation), E Lipton (by invitation), and P Patterson (by invitation), *Departments of Pharmacology and Pediatrics, University of Illinois College of Medicine, Chicago*  
Efficacy of atropine in children
- 3 Charles M Gruber and Goldie Freedman Keyser (by invitation), *Department of Pharmacology, Jefferson Medical College, Philadelphia*  
The effects of 1 amino-1-phthalidylpropane hydrochloride on excised and intact intestine and uterus
- 4 Julius M Coon and Paul R Salerno (by invitation), *University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago*  
A pharmacologic comparison of hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP) with physostigmine and neostigmine
- 5 H Walter Jones, Jr (by invitation), Bertram J Meyer (by invitation) and Leonard Karel, *Toxicology Section, Medical Division, Army Chemical Center, Md*  
The relationship of cholinesterase inhibiting activity to the acute toxicity of some organic phosphorus compounds
- 6 H E Himwich and A M Freedman (by invitation), *Medical Division, Army Chemical Center, Md*  
Di isopropyl fluorophosphate (DFP) Site of

injection and variation in response (Physiol)

### PHARMACOLOGY A

Wednesday, March 17, 1 45 p m

ROOM E, CONVENTION HALL

#### Curare and Anti-Curare-like Compounds

- 1 K K Kimura (by invitation) and K R Unna, *Department of Pharmacology, University of Illinois College of Medicine, Chicago*  
Methods for the evaluation of compounds with curare like action
- 2 Roger F Varney (by invitation), Charles R Linegar and Horace A Holaday (by invitation), *Biological and Chemical Laboratories, E R Squibb & Sons, New Brunswick*  
The rabbit "Head drop" method for the biological assay of curare and its alkaloids
- 3 E F Van Maanen (introduced by Otto Krayer), *Department of Pharmacology, Harvard Medical School, Boston*  
A comparison of curare alkaloids
- 4 E L McCawley, *the Laboratories of Pharmacology and Toxicology and the Laboratory of Neuro Anatomy, Yale University School of Medicine, New Haven*  
Some aspects of the action of curare on the central nervous system
- 5 G M Everett (introduced by R K Richards), *Department of Pharmacology, Abbott Research Laboratories, North Chicago*  
Studies on the toxicity and metabolism of d-tubocurarine
- 6 M H Pelletier (by invitation) and David Fielding Marsh, *Department of Pharmacology, West Virginia University School of Medicine, Morgantown*  
Synthetic curare compounds II d N Methylisochondrodendrine iodide and d N-Methyl O methyl isochondrodendrine iodide
- 7 David Fielding Marsh, Clark K Sleeth (by invitation), and Eldon B Tucker (by invitation), *Departments of Pharmacology and Medicine, West Virginia University School of Medicine, Morgantown*  
Synthetic curare compounds III d N Methylchondrodendrine iodide and d-N Methyl O-methyl chondrodendrine iodide
- 8 John C Burke (by invitation) and Charles R Linegar, *Pharmacological Development Division, E R Squibb & Sons, New Brunswick*  
Pharmacological studies of myanesin and curare
- 9 H A Walker (by invitation), Arthur P Richardson, P Loeb (by invitation) and J Perog (by invitation), *Department of Pharmacology,*

*Emory University School of Medicine, Emory University, Georgia, and Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick*

The paralytic and lethal action of myanesin, pentobarbital and combinations of these agents in mice

- 10 **James L Morrison, Arthur P Richardson and Harry A Walker** (*by invitation*), *Department of Pharmacology, Emory University, Georgia*  
Rate of disappearance from the blood and distribution in tissues of 3-(Orthotoloyl) 1,2-propanediol (Myanesin)

- 11 **Mitchell Zweig** (*by invitation*), **F Steigmann**, and **Karl A Meyer** (*by invitation*), *Helton Institute for Medical Research of Cook County Hospital and the Department of Surgery, Northwestern University School of Medicine*  
The effect of tetraethyl ammonium chloride on gastric secretion and motility

- 12 **Charles J Kensler** (*introduced by McKeen Cattell*), *Department of Pharmacology, Cornell University Medical College*  
The antitumor activity of congo red and related compounds

### PHARMACOLOGY B

Wednesday March 17, 1:45 p.m.

ROOM D, CONVENTION HALL

Joint Session, Biometrics Section,  
American Statistical Association,  
and the Pharmacological Society

- 1 **E M Jellinek** (*introduced by C I Bliss*), *Department of Applied Physiology Yale University, New Haven*

Experimental bromism in man

- 2 **Graham Chen, A C Bratton, Jr and Charles Ensor** (*by invitation*), *Research Laboratories, Parke, Davis & Company, Detroit*

The joint antihistaminic effect and acute toxicity of adrenalin and benadryl HCl

- 3 **Rex G Fluharty** (*introduced by Lloyd C Miller*), *Radioactivity Center, Massachusetts Institute of Technology*

Statistical problems involved in the use of radioactive tracers in pharmacology

- 4 **J T Litchfield, Jr, and F Wilcoxon** (*by invitation*), *Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford*

A simplified method of evaluating dose effect experiments

- 5 **R H Noel and M A Brumbaugh** (*introduced by Lloyd C Miller*), *Bristol Laboratories, Inc, Syracuse*

Some applications of sequential analysis

### Federation "Mixer"

Wednesday, March 17, 9:00 p.m.

ARENA, CONVENTION HALL

### PHARMACOLOGY A

Thursday, March 18, 9:00 a.m.

ROOM I, CONVENTION HALL

### Barbiturates and Analgesics

- 1 **Walter M Booker, David M French, Pedro A Molano, and Cecil Rhodes** (*introduced by A H Maloney*), *Department of Pharmacology, College of Medicine, Howard University, Washington*

Re evaluation of the effectiveness of metrazol as an analeptic agent in barbiturate depression

- 2 **V V Cole, S H Hopper** (*by invitation*), and **H R Hulpacu**, *Depts of Pharmacology and Public Health, Indiana Univ School of Medicine, Indianapolis*

Effects of surface active agents on the activity of pentobarbital and barbital

- 3 **Marshall R Warren** (*by invitation*), **Charles R Thompson** (*by invitation*), and **Harold W Werner**, *Pharmacology Department, Research Labs, The Wm S Merrell Co, Cincinnati*  
Hypnotic properties and toxicity of 2 ethyl 3 propylglycidamide

- 4 **A R Kelly** (*by invitation*), **F E Shideman** and **B J Adams** (*by invitation*), *Dept of Pharmacology, University of Michigan, Ann Arbor*  
Comparison of blood levels of thiopental (Pentothal), 5 allyl 5-(1-methylbutyl) 2 thiobarbituric acid (Surital) and thioethamyl in the dog

- 5 **F E Shideman, A R Kelly** (*by invitation*), **L E Lee** (*by invitation*), **V F Lowell** (*by invitation*) and **B J Adams** (*by invitation*), *Department of Pharmacology, University of Michigan and Ypsilanti State Hospital, Ypsilanti, Mich*

The effect of hepatic dysfunction in man on the duration of action of thiopental (Pentothal)

- 6 **Leo R Goldbaum** (*by invitation*) and **Paul K Smith**, *Department of Pharmacology, George Washington University School of Medicine, Washington*

The binding of barbiturates by human and bovine serum albumin

- 7 **W J R Camp, V A Gant** (*by invitation*) and **John F Polli** (*by invitation*), *Dept of Toxicology and Pharmacology, University of Illinois College of Medicine, Chicago*

The use of ultraviolet rays in a screening test

for some barbiturates and in reading Gutzert papers

- 8 D D Bonnycastle and Jacob Molland (by invitation), Departments of Pharmacology, Yale School of Medicine, and University of Oslo, Norway

An examination of potentiating action of aspirin upon codeine in raising the pain threshold

### PHARMACOLOGY B

Thursday, March 18, 9 00 a m

ROOM 9, CONVENTION HALL

#### Chemotherapeutic Agents

- 1 Thomas H Maren and Gilbert F Otto (introduced by E K Marshall, Jr), Department of Pharmacology, Johns Hopkins University School of Medicine and Department of Parasitology, Johns Hopkins School of Hygiene and Public Health, Baltimore

The distribution, excretion, and blood levels of antimony following administration of antimonials to various mammalian species

- 2 Ralph G Smith, James Y P Chen (by invitation) and Marguerite Magee (by invitation), Department of Pharmacology, Tulane University School of Medicine, New Orleans

The distribution and excretion of antimony following administration of antimony potassium tartrate and neostibosan

- 3 Maxwell Schubert and Evelyn Goldberg (by invitation), Department of Therapeutics, New York University College of Medicine

Comparison of therapeutic values of several antimonials in experimental schistosomiasis mansonii in mice

- 4 Harold N Wright, Elizabeth M Cranston, Wayne A Chadbourn (by invitation), Ashton C Cuckler (by invitation), Dominic DeGuisti (by invitation) and Raymond N Bieter, Department of Pharmacology, University of Minnesota Medical School, Minneapolis

Rosaniline base (CI 677) as a prophylactic in schistosoma mansonii infections in mice

- 5 Clara S Genther (by invitation), Wanda Squires (by invitation), Rochelle Fradkin (by invitation), and L H Schmidt, Christ Hospital Institute of Medical Research, Cincinnati

Malaria chemotherapy 1 The response of trophozoite-induced infections with *Plasmodium cynomolgi* to various antimalarial drugs

- 6 L H Schmidt, Rochelle Fradkin (by invitation), Wanda Squires (by invitation), and Clara S Genther (by invitation), Christ Hospital Institute of Medical Research, Cincinnati

Malaria chemotherapy 2 The response of sporozoite induced infections with *Plasmodium cynomolgi* to various antimalarial Drugs

- 7 David Lehr, Department of Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York

Low toxicity and high therapeutic efficacy of combined sulfonamides

- 8 Celia White Tabor (by invitation), Judith Bailly (by invitation) and Paul K Smith, Department of Pharmacology, George Washington University School of Medicine, Washington

Some metabolic products of para aminobenzoic acid

- 9 E Leong Way, Rowena Weiss (by invitation), Donald L Howie (by invitation) and Paul K Smith, Department of Pharmacology, George Washington University School of Medicine, Washington

The fate of p aminosalicylic acid in the animal body

- 10 W T McClosky, M I Smith and J E G Frias (by invitation), Division of Physiology, National Institute of Health, Bethesda, Md

The action of p aminosalicylic acid (PAS) in experimental tuberculosis

- 11 S H Hopper (by invitation), V V Cole, H R Hulpieu, and H A Raidt (by invitation), Departments of Public Health, Pharmacology, and Microbiology, Indiana University School of Medicine, Indianapolis

Opposite effects in vitro and in vivo of a surface active agent on mycobacterium tuberculosis

### PHARMACOLOGY C

Thursday, March 18, 9 00 a m

ROOM 1, CONVENTION HALL

#### Agents Affecting the Blood and Vascular System

- 1 Anthony M Ambrose, Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U S Department of Agriculture, Albany

The effect of rutin on blood pressure in dogs and rabbits

- 2 Walter Freyburger (by invitation), Luis R Capo (by invitation), and Gordon K Moe, Department of Pharmacology, the University of Michigan, Ann Arbor

The effect of tetraethylammonium on the pressor response to anoxia and asphyxia

- 3 Donald C Kunze (by invitation), J Richard R Bobb (by invitation) and Harold D Green, Department of Physiology and Pharmacology,

*Bowman Gray School of Medicine of Wake Forest College, Winston Salem*

Visomotor activity of NU-1683 and other drugs upon the rat meso appendix

- 4 **D M Green and M Glover** (by invitation), *School of Medicine, University of Washington, Seattle*

Factors influencing the hypertensive action of desoxycorticosterone

- 5 **Joseph G Bird** (introduced by John C Krantz, Jr.), *Department of Pharmacology, University of Maryland School of Medicine, Baltimore*

Prolonged depressor effect of water soluble organic nitrates

- 6 **Albert J Begany** (by invitation) and **Joseph Seifter**, *Wyeth Institute of Applied Biochemistry, Philadelphia*

Studies on a heparin-like compound, algin

- 7 **David W Fassett and E Sterling Nichol** (by invitation), *Cardiology Service, James M Jackson Memorial Hospital, Miami*

Absorption and potency of small divided doses of dicumarol in man

- 8 **Rose Marie Carlson** (by invitation) and **Lloyd D Seager**, *Department of Pharmacology, Woman's Medical College of Pennsylvania, Philadelphia*

The acute toxic effects of dicumarol

- 9 **Robert V Brown**, *Division of Pharmacology, University of Tennessee, Memphis*

Quantitative epinephrine pressor effects (Physiol)

- 10 **E William Ligon, Jr** (by invitation), **Robert A Madden** (by invitation), **Paul L Davis** (by invitation) and **Paul K Smith**, *Department of Pharmacology, George Washington University School of Medicine, Washington*

Absorption of silylates from intestinal loops in the dog

- 11 **Bernard B Brodie and Julius Axelrod** (by invitation), *Department of Biochemistry, New York University College of Medicine, and New York University Research Service, Goldwater Memorial Hospital, and Laboratory of Industrial Hygiene, New York*

The physiological disposition of acetophenetidin (p ethoxyacetamide) in man

- 12 **R R Sonnenschein, R Jamison, L Lovseth, W Cassels** (by invitation) and **A C Ivy**, *Departments of Clinical Science and Anesthesia, University of Illinois College of Medicine, Chicago*

The mechanism of nitrous oxide analgesia (Physiol)

## PHARMACOLOGY A

Thursday, March 18, 1959 p m

Room E, CONVENTION HALL

### Cardiac and Related Agents

- 1 **R P Walton, M F Patton** (by invitation), **H P Jones** (by invitation) and **J S Leary** (by invitation), *Department of Pharmacology, Medical College of South Carolina, Charleston*  
Comparative increase in ventricular contractile force produced by several cardiac glycosides

- 2 **Mark Nickerson and George M Nomaguchi** (by invitation), *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City*

Dibenzamine protection against cyclopropane (pinphrine) cardiac arrhythmias

- 3 **K I McVillie**, *Dept of Pharmacology, McGill University, Montreal*

The anti fibrillation action of papaverine and its value in cardiac resuscitation after chloroform adrenalin ventricular fibrillation

- 4 **Otto Kraye and Alfred Farah**, *Department of Pharmacology, Harvard Medical School, Boston*

Action of cysteine and of dimercaptopropanol in heart failure caused by sodium bismuth tartrate

- 5 **N J Garman** (introduced by E L McCawley), *Laboratory of Pharmacology and Toxicology, Yale University School of Medicine, New Haven*

Toxicity and cardiotoxic activity of antibiotic lactones and synthetic analogs on the isolated frog heart

- 6 **A Cameron** (by invitation), **M Laskey** (by invitation), **B Craver**, *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc, Summit, N J*

Actions and minimally effective doses of 17 common drugs on perfused hearts of 5 species

- 7 **Maynard B Chenoweth and Solomon Garb** (by invitation), *Department of Pharmacology, Cornell Univ Medical College, New York, N Y*

The electrogram of the pupillary muscle

- 8 **A Farah and E Szmuszkoviez** (introduced by James M Dille), *Departments of Pharmacology, University of Washington Medical School, Seattle, and the American University of Beirut, Lebanon*

Some factors influencing the activity of cardiac glycosides in the rat



**PHARMACOLOGY BUSINESS MEETING***Thursday, March 18, 4 15 p m*

ROOM E, CONVENTION HALL

**PHARMACOLOGY DINNER***Thursday, March 18, 7 00 p m*

HOTEL HADDON HALL

**PHARMACOLOGY B***Thursday, March 18, 1 45 p m*

ROOM 9, CONVENTION HALL

- 1 Salab A Abdel Tawab (introduced by John C Krantz, Jr), Department of Pharmacology, University of Maryland School of Medicine, Baltimore

The pharmacology of organic thiocyanates

- 2 Ewart A Swinyard (by invitation) and James E P Toman, Departments of Pharmacology, Pharmacy, and Physiology, University of Utah, Salt Lake City

Effects of alterations in body temperature on properties of convulsive seizures in rats

- 3 Avram Goldstein (introduced by Otto Kraye), Department of Pharmacology, Harvard Medical School, Boston

Inhibitors of purified human plasma cholinesterase

- 4 Rafael Mendez and Ernesto Sodi (by invitation), Department of Physiology and Pharmacology of the National Institute of Cardiology, Mexico City

The effect of some glycerin esters upon the blood pressure, uterus, and respiration

- 5 E Herrold (by invitation), H Hays (by invitation), G Holmquist (by invitation), B Richards (by invitation), E Oppenheimer, Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc, Summit, N J

The water soluble I glucoside of desoxycorticosterone acetate, its adrenal cortical activity in adrenalectomized dogs

- 6 Victor A Drill and B Frame (by invitation), Department of Pharmacology, Yale University School of Medicine, New Haven

Antidiuretic activity of liver extracts and of urine from patients with hepatic cirrhosis

- 7 Douglas S Riggs (introduced by Otto Kraye), Department of Pharmacology, Harvard Medical School and The Thyroid Clinic, Massachusetts General Hospital

Elevation of serum protein bound iodine after large doses of radio active iodine

- 8 J K W Ferguson and E A Sellers, Department of Pharmacology, University of Toronto

Effect of various compounds containing iodine and other halogens on thiouracil goitre

- 9 D G Wenzel (by invitation), O S Orth, R T Capps (by invitation), and A H Uhl (by invitation), Departments of Pharmacy and Pharmacology, University of Wisconsin, Madison

Actions of podophyllum derivatives

- 10 Allan D Bass, Pharmacology Department, Syracuse University

Response of experimental lymphoma to certain organic sulfides, sulfones and chlorides

**PHARMACOLOGY BUSINESS MEETING***Thursday, March 18, 4 15 p m*

ROOM E, CONVENTION HALL

**PHARMACOLOGY DINNER***Thursday, March 18, 7 00 p m*

HOTEL HADDON HALL

**PHARMACOLOGY C***Thursday, March 18, 1 45 p m*

ROOM 1, CONVENTION HALL

**Drug Toxicity**

- 1 Geoffrey Woodward (by invitation), Bernard Davidow (by invitation), and Arthur A Nelson, Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington

Effects observed in dogs following the prolonged feeding of DDT and its analogues

- 2 Lloyd W Hazelton and Emily Godfrey (by invitation), Hazelton Laboratories, Falls Church, Va

Pharmacological actions of o,o diethyl o p nitrophenyl thiophosphate

- 3 Ernest C Hagan and Geoffrey Woodard (introduced by Arnold J Lehman), Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington

Toxicity of o,o diethyl o p nitrophenyl thiophosphate (Parathion)

- 4 Kenneth P DuBois, John Doull (by invitation), and Julius M Coon, University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago

Toxicity and mechanism of action of p nitrophenyl-diethyl thionophosphate (E605)

- 5 **Jack L Radomski, Geoffrey Woodard, Carter D Johnston** (*introduced by Arnold J Lehman*), *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
Toxicological properties of the causative agent of canine hysteria
- 6 **W F von Oettingen, C C Powell** (*by invitation*), **N E Sharpless** (*by invitation*) and **L J Pecora** (*by invitation*), *Laboratory of Physical Biology, National Institute of Health, Bethesda, Md*  
Comparative studies of the toxicity and physiological action of chlorinated methanes with reference to their physical and chemical characteristics
- 7 **Edwin P Laug**, *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
Tissue distribution of a toxicant following oral ingestion of benzene hexachloride by rats
- 8 **Bernard Davidow, Ernest C Hagan and Geoffrey Woodard** (*introduced by Arnold J Lehman*), *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
A comparison of the fat storage of the isomers of benzene hexachloride in rats
- 9 **Leonard Karel**, *Toxicology Section, Medical Division, Army Chemical Center, Md*  
A potential rodenticide—"Fanyline"
- 10 **Elliott A Maynard, William L Downs, and Harold C Hodge** (*introduced by Harvey B Haag*), *Department of Pharmacology, University of Rochester, N Y*  
Preliminary data on rat feeding with beryllium
- 11 **Harold C Hodge, Elliott A Maynard and William L Downs** (*introduced by Harvey B Haag*), *Department of Pharmacology, University of Rochester, N Y*  
Certain aspects of the acute toxicity of beryllium following intraperitoneal injection
- 12 **Charles W LaBelle and Martha Reid Cucci** (*introduced by Harvey B Haag*), *Department of Pharmacology, University of Rochester, N Y*  
Preliminary studies in the toxicity of beryllium. The effect of intratracheal injections in experimental animals
- 13 **George F Sprague, Alton G Pettengill and Herbert E Stokinger** (*introduced by Harvey B Haag*), *Department of Pharmacology, University of Rochester, N Y*  
Initial studies of the inhalation toxicity of beryllium sulfate
- 14 **Sidney Laskin, Robert A N Turner and Herbert E Stokinger** (*introduced by Harvey B*

*Haag*), *Department of Pharmacology, University of Rochester, N Y*  
Analysis of dust and fume hazards in a beryllium plant

## PHARMACOLOGY BUSINESS MEETING

Thursday, March 18, 4 15 p m

ROOM L, CONVENTION HALL

## AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Annual Dinner

Thursday, March 18, 7 00 p m

HOTEL HADDON HALL

## PHARMACOLOGY

Motion Pictures

Thursday, March 18, 8 00 p m

ROOM C, CONVENTION HALL

Program on page 314

## PHARMACOLOGY A

Friday, March 19, 9 00 p m

ROOM E, CONVENTION HALL

## Histamine, Anti-histamine and Spasmolytic Agents

- 1 **Donald L Cook** (*by invitation*), **W E Hamburger and Martin M Winbury** (*by invitation*), *Pharmacology Laboratories, Research Department, G D Searle & Co, Chicago*  
Inhibition of histamine hypotension by certain heterocyclic substituted ethylamines in the cat
- 2 **Homer B Freese** (*by invitation*), **W E Hamburger and Patricia M Michiels** (*by invitation*), *Pharmacology Laboratories, Research Department, G D Searle & Co, Chicago*  
The effectiveness of certain substituted ethylamines against histamine spray induced fatality in the guinea pig
- 3 **Julio C Castillo** (*introduced by Edwin J de Beer*), *The Wellcome Research Laboratories, Tuckahoe*  
The anaphylactic guinea pig trachea and its response to antihistamine and bronchodilator drugs

- 4 J Smith (by invitation), A Cameron (by invitation), B Craver, Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc, Summit, N J

Method for kymographic recording of CSF pressure, effects of histamine, antisthine, PBZ, et al, thereon

- 5 Herbert Tabor and Sanford M Rosenthal, Division of Physiology, National Institute of Health, Bethesda, Md

Urinary excretion of histamine following oral administration, employing an improved colorimetric method

- 6 Lillian Alonso (by invitation), Maxine Adams (by invitation), Louise Goddard (by invitation), Marion Jaeger (by invitation), and J T Litchfield, Jr, Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford

Mode of action of antihistaminic agents

- 7 George W Stavratsky, Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada

Potentiation of the effect of antihistaminic agents by iron compounds

- 8 Annette LaBelle and Richard Tislow (introduced by O Krayer), Biological Research Laboratories, Schering Corporation, Bloomfield

Pharmacological properties of trimeton, a new antihistaminic compound

- 9 B Craver, W Barrett (by invitation), A Cameron (by invitation), H Hays (by invitation), G Holmquist (by invitation), A Mackenzie (by invitation), J Smith (by invitation), Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc, Summit, N J

Pharmacological actions of 2 phenylbenzylaminomethyl imidazoline (Antisthine)

- 10 Robert H Dreisbach and Nai Chu (Fellow, American Bureau for Medical Aid to China Inc, by invitation), Dept of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco

Procaine as an antiallergic agent

- 11 A J Glazko, W A Dill (by invitation), and D A McGinty, Research Laboratories, Parke, Davis and Co, Detroit

Distribution and excretion of benadryl ( $\beta$ -dimethylaminoethyl benzhydrol ether) (Biochem)

- 12 T C Barnes, Department of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia

The adrenergic properties of antihistaminic drugs determined by electrical measurements (Physiol)

## PHARMACOLOGY B

Friday, March 19, 9 00 a m

Room 9, CONVENTION HALL

### Cardiac and Related Agents

- 1 Elwood L Foltz, Sau Ki Wong and James E Eckenhooff (introduced by Carl F Schmidt), Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia

Effects of certain "cardiac stimulant" drugs on coronary circulation and cardiac oxygen metabolism

- 2 Murray Finkelstein (by invitation) and Oscar Bodansky, Dept of Pharmacology, Cornell University Medical College, New York

Effect of scilthroside on the respiration of cat heart muscle

- 3 Albert Wollenberger (introduced by Otto Krayer), Harvard Medical School, Boston

Effect of ouabain and digoxin on the energy-rich phosphate store of the heart

- 4 Paul R Saunders (by invitation), J Leyden Webb (by invitation), and Clinton H Thienes, Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles

Effect of quindine on the metabolism of the rat heart

- 5 Harry K Iwamoto and Frederick K Bell, (introduced by John C Krantz, Jr), Department of Pharmacology, School of Medicine, Univ of Maryland, Baltimore

The Baljet reaction and pharmacodynamic studies of diginin and gitoginin

- 6 Walter Modell, Nathaniel T Kwit (by invitation), Conrado Dayrit (by invitation), S J Shane (by invitation) and Harry Gold, Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York

Comparison of digitalis glycosides with their genins in man

- 7 Nathaniel T Kwit (by invitation), Walter Modell, Lawrence W Hanlon (by invitation), Joseph G Benton (by invitation), Elaine W Cotlove (by invitation), Morris Pearlmutter (by invitation), Sidney M Greenberg (by invitation), Milton L Kramer (by invitation), and Harry Gold, Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York

The daily dose of the mercurial diuretic and the maintenance dose to control congestive failure

- 8 **S J Shane** (*by invitation*), **Conrado Dayrit** (*by invitation*), **Joseph G Benton** (*by invitation*), **Elaine W Cotlove** (*by invitation*), **Lawrence W Hanlon** (*by invitation*), **Walter Modell**, and **Harry Gold**, *Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York*

Onset and duration of action of quinidine in the heart after oral administration in man

- 9 **Conrado Dayrit** (*by invitation*), **Eduardo Faraco** (*by invitation*), **Nathaniel T Kwit** (*by invitation*), **Walter Modell**, and **Harry Gold**, *Department of Pharmacology of Cornell University Medical College, Cardiovascular Research Unit of the Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York*

Speed of action of thevetin after intravenous injection in man

### PHARMACOLOGY C

Friday, March 19, 9 00 a m

ROOM 1, CONVENTION HALL

- 1 **R M Whitrock** (*by invitation*), **H L Tieche** (*by invitation*), and **M H Seevers**, *Department of Pharmacology, University of Michigan, Ann Arbor*

The effect of "denervation" on the response of intestinal fistulae to several drugs

- 2 **Ralph Uber** (*by invitation*), **Patricia Collins Cowdery** (*by invitation*) and **George E Farrar, Jr**, *Department of Medicine, Temple University School of Medicine, Philadelphia*

The spreading of Evan's blue injected intradermally

- 3 **Ralph W Brauer** and **Rita L Pessotti** (*introduced by Chapman Reynolds*), *Department of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State University, New Orleans*

The mechanism of the extraction of bromosulfalein from blood plasma by the liver

- 4 **H Ward Smith** (*introduced by G H W Lucas*), *Department of Pharmacology, University of Toronto*

The specificity of the determination of alcohol in biological fluids

- 5 **Edward B Truitt, Jr** (*introduced by John C Krantz, Jr*), *Department of Pharmacology, University of Maryland, School of Medicine, Baltimore*

The quantitative estimation of theophylline in blood

- 6 **R A Woodbury**, **George P Child** (*by invitation*), and **R Torpin** (*by invitation*), *Division of Pharmacology, University of Tennessee, Memphis, and Departments of Pharmacology and Obstetrics and Gynecology, University of Georgia School of Medicine, Augusta*

Oxytocic drugs and dysmenorrhea

- 7 **Joseph Thomas Roberts**, *University of Arkansas School of Medicine, Little Rock, Arkansas and Gallinger Municipal Hospital, Washington*

The effect of globulin insulin and protamine zinc insulin on the diurnal blood sugar curve and the daily glycosuria of diabetics, and the clinical use of globin insulin

- 8 **A J Plummer** (*introduced by George L Mason*), *Department of Pharmacology, Boston University School of Medicine*

Blood and urine levels of theophylline after intravenous injection (Physiol)

- 9 **Leon A Hoppel** and **Virginia T Porterfield** (*introduced by B L Horecker*), *Laboratory of Physical Biology, National Institute of Health, Bethesda, Md*

Enzymatic cleavage of organic halides (Biochem)

- 10 **F Co Tui**, **J H Mulholland** (*by invitation*) and **N Knox** (*by invitation*), *Department of Surgery, New York University College of Medicine*

Protein depletion syndrome and responses to hyperclimentation

### PHARMACOLOGY

#### Papers Read by Title

- 1 **Benedict E Abreu** and (*by invitation*), **Grant W Liddle**, **Arthur L Burks**, **Alexander Simon**, **Violette Sutherland** and **Gilbert S Gordon**, *Division of Pharmacology and Experimental Therapeutics, and Psychiatry, University of California Medical School, San Francisco*

Effects of imphetamine, dihydroergotamine, and methadon on human cerebral blood flow and oxygen uptake

- 2 **Shannon C Allen** and **Talbot G Mortarotti** (*by invitation*), *Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U S Department of Agriculture, Albany, Calif*

The effect of rutin on oxygen toxicity in rats

- 3 **Anthony M Ambrose**, *Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U S Department of Agriculture, Albany, Calif*

The effect of rutin and quercetin on scorbutic guinea pigs

- 4 **Hamilton H Anderson**, **Herbert G Johnstone**

- (by invitation), and A Pena Chavarria (by invitation), *University of California Medical School, San Francisco*, and Hospital San Juan de Dios, San José de Costa Rica, A C Parasitocidal activity of thiorsemites in man
- 5 Arthur L Bachelor and Henry W Elliott (introduced by Benedict E Abreu), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
The action of methadon upon the respiration of rat diaphragm and liver and kidney cortex slices
- 6 T R W Barnes (introduced by Charles H Hine), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
The toxicity of polyallyl alcohol
- 7 W Barrett, A Cameron, N Hansen, E Herrold, A Mackenzie, B Richards, F Roth, J Smith (all by invitation), (introduced by F F Yonkman), *Division of Pharmacology, Research Department, Ciba Pharmaceutical Products, Inc, Summit, N J*  
Toxicological and pharmacological data concerning 2-benzyl imidazole hydrochloride (Priscol)
- 8 Harry Beckman, *Marquette University School of Medicine*  
Infectivity of sporozoites of *Plasmodium Cathemerium 3H2* exposed *in vitro* to hen and canary bloods
- 9 Harold F Chase and Bani K Bhattacharya (by invitation), *Departments of Pharmacology and Surgery, Western Reserve University and The University Hospitals of Cleveland, Cleveland*  
Prolongation of curare action with a peanut oil and beeswax vehicle
- 10 Yin-ch'ang Chin (introduced by Hamilton H Anderson), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Effect of some derivatives of subtilin on tubercle bacilli and rabbit leukocytes *in vitro*
- 11 F Co Tui, V Hollander, A S Keston, J H Mulholland, *Department of Surgery, New York University College of Medicine*  
Total body water by D O tracer study in protein depletion
- 12 John Emerson Davis, *University of Arkansas School of Medicine, Little Rock*  
Failure of anti histaminic drugs to antagonize the anemia produced by fat plus choline
- 13 M Edward Davis (by invitation), Nicholas W Fugo, and Evelyn Ai-Feng Yu (by invitation), *Department of Obstetrics and Gynecology and the Department of Pharmacology, University of Chicago and The Chicago Lying-in Hospital*  
Some observations on the non specific choline esterase in the human female
- 14 Victor A Drill, *Department of Pharmacology, Yale University School of Medicine, New Haven*  
Relation of meat intoxication in Eck-fistula dogs to hepatic dysfunction
- 15 Frank G Everett (introduced by Norman David), *Department of Pharmacology, University of Oregon Medical School, Portland*  
Comparison of depth of local anesthesia obtained with 2% and 4% procaine solutions
- 16 Chalmers L Gemmill, *Department of Pharmacology, University of Virginia, Medical School, Charlottesville*  
Inhibitory effect of stilbamidine, guanidine and arginine on glycolysis
- 17 Alvin S Hambly, Jr (introduced by Hamilton H Anderson), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Chloroquine in human giardiasis
- 18 Carroll A Handley and Marguerite LaForge (by invitation), *Dept of Pharmacology, Baylor University College of Medicine, Houston*  
Some extra cardiac effects of digitalis
- 19 Eder L Hansen and Rachael K Reed (introduced by Hamilton H Anderson), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Action of peroxides on endameba histolytica
- 20 Roy G Herrmann (by invitation) and Kenneth P DuBois, *University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago*  
The action of p dimethylaminobenzenediazo sodium sulfonate on carbohydrate metabolism
- 21 Charles H Hine and Thomas Nathaniel Burbridge (by invitation), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
The effects of 2,4 dinitrophenol and thioracil on chronic methanol poisoning
- 22 Helen L Holland (by invitation) and E G Gross, *Department of Pharmacology, School of Medicine, Iowa City*  
Two new analgesics
- 23 K Hwang (by invitation) and K R Unna, *Department of Pharmacology, University of Illinois College of Medicine, Chicago*  
The effect of curare on the esophagus of the dog
- 24 Hal P James and Ernest W Page (introduced

- by C H Hine), *Divisions of Obstetrics and Gynecology and of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Influence of some analgetics of obstetrical importance upon respiration of mature human placenta *in vitro*
- 25 **Norman W Karr** (introduced by Norman A David), *Department of Pharmacology, University of Oregon Medical School, Portland*  
Circulatory and adrenolytic actions of DHE and DHO
- 26 **A C Kirchhof**, *The Division of Anesthesiology, University of Oregon Medical School, Portland*  
Further studies on synthetic analgesics
- 27 **C D Leake**, *University of Texas Medical Branch, Galveston*  
Molecular architecture of cells
- 28 **David Lehr**, *Department of Pharmacology, New York Medical College, Floucr and Fifth Avenue Hospitals, New York*  
Studies on the toxicity and pharmacology of some synthetic antispasmodics
- 29 **Go Lu** (Fellow, American Bureau for Medical Aid to China Inc, by invitation), *Department of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco*  
Comparative effects of sparteine and quindine on isolated frog heart
- 30 **David I Macht and Thomas Hoffmaster** (by invitation), *Division of Pharmacology, Laboratories of Sinai Hospital, Baltimore*  
Influence of benadryl and pyribenzamine on the neuromuscular system of rats
- 31 **W A McOmie** (introduced by Hamilton H Anderson), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School and College of Pharmacy, San Francisco*  
Acute vapor toxicity to mice and local irritant effects in the rabbit of methyl pentadiene
- 32 **Mark Nickerson**, *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City*  
Effect of procaine on cyclopropane-epinephrine cardiac arrhythmias
- 33 **Carl C Pfeiffer, Elizabeth H Jenney** (by invitation) and **I Gersh**, *Depts of Pharmacology and Pathology, University of Illinois College of Medicine, Chicago*  
Chronic intoxication of the CNS produced by the methadon sidechain
- 34 **Frederick S Philips, Maynard B Chenoweth, and Carlton C Hunt** (by invitation), *Departments of Pharmacology, Cornell Univ Medical College and the Sloan-Kettering Institute for Cancer Research, New York*  
Studies on the toxicology of podophyllotoxin and related substances
- 35 **Raymond W Pickering and Richard C Burnett** (introduced by Benedict E Abreu), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Spasmolytic and other pharmacologic properties of  $\beta$  piperidinoethyl phenyl  $\alpha$  thienyl glycolate
- 36 **Harry J Pratt** (introduced by R Beutner), *Department of Pharmacology, Hahnemann Medical College, Philadelphia*  
Abolition of extra systoles in the perfused isolated rabbit heart by procaine and monocaine hydrochlorides
- 37 **R R Rollins** (introduced by Hamilton H Anderson), *Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco*  
Comparative intragastric and local toxicities of tert butyl hydrogen peroxide, di tert butyl peroxide, and 2,2 bis di-tert butyl peroxobutane
- 38 **Edward Saxey** (by invitation) and **Nilkanth M Phatak**, *Department of Pharmacology, University of Oregon Medical School, Portland*  
Use of oxygen consumption method for measuring antithyroid activity of n propyl thiouracil, thiouracil and 2 aminothiazole in rats
- 39 **F C Shideman, A R Kelly** (by invitation), and **B J Adams** (by invitation), *Department of Pharmacology, University of Michigan, Ann Arbor*  
Blood levels of thiopental (Pentothal) following repeated intravenous administration to the dog
- 40 **Robert Tarail and W Lane Williams** (introduced by R N Bieter), *Departments of Anatomy and Medicine, University of Minnesota*  
Some effects of ouabain and/or calcium in mice
- 41 **Ronald M Thompson** (by invitation) and **Julius M Coon**, *Dept of Pharmacology, University of Chicago*  
Effect of adrenolytic agents on the response to pressor substances in the domestic fowl
- 42 **Abraham Wikler**, *Research Department, U S Public Health Service Hospital, Lexington, Ky*  
Reactions of chronic decorticated dogs during a cycle of addiction to methadon
- 43 **Robert H Wilson, J C Lewis and E M Humphreys** (by invitation), *Pharmacology Division and the Western Regional Research Laboratory of the Bureau of Agriculture and*

- Industrial Chemistry, U S D 4, Albany, Calif*  
 Subtilin in blood after parenteral administration
- 44 **Martin M Winbury and John Love** (introduced by W E Hambourger), *Lehigh Valley Laboratories, Easton, Pa*  
 The effect of ANTU on the body temperature in the rat
- 45 **S Loewe**, *Department of Pharmacology, University of Utah School of Medicine, Salt Lake City*  
 Anticonvulsant actions of trimethadione phenobarbital and trimethadione diphenylhydantoin combinations
- 46 **Alfred Leimdorfer**, *Department of Pharmacology, University of Illinois, College of Medicine, Chicago*
- Influence of amdone, morphine and strychnine on the Straub-tail test of white mice
- 47 **Emmett B Carmichael and Walter H Johnson** (by invitation), *Biochemistry Department, Medical College of Alabama, Birmingham*  
 The LD<sub>50</sub> of pentobarbital in nursed and un-nursed newborn rats (Biochem)
- 48 **T C Barnes**, *Dept of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia*  
 Phase-boundary potentials of some analogs of prostigmine (Physiol)
- 49 **John V Scudi, John F Reinhard** (by invitation) and **N B Dreyer** (by invitation), *Nepera Chemical Co, Yonkers, and the Department of Pharmacology, University of Vermont*  
 Pharmacological characteristics of neohetramine, a new antihistaminic drug (Biochem)

## THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

### THIRTY-THIRD ANNUAL MEETING

#### PATHOLOGY A

Tuesday, March 16, 9 00 a m

ROOM 15, CONVENTION HALL

#### Injury, Repair

- 1 **John W Rebuck and Elizabeth A Monaghan** (introduced by F W Hartman) *Department of Anatomy, University of Minnesota, Minneapolis, and Department Labs, Henry Ford Hospital, Detroit*  
 Peroxidase in the lymphocytes of man in acute inflammation
- 2 **Edwin W Schultz**, *Department of Bacteriology and Experimental Pathology, School of Medicine, Stanford University, Calif*  
 Structure and repair of the olfactory mucosa in rhesus monkeys
- 3 **Louise Pearce**, *Rockefeller Institute for Medical Research, Princeton*  
 Hereditary osteopetrosis of the rabbit
- 4 **Robert S Haukohl** (introduced by W A D Anderson), *Department of Pathology and Bacteriology, Marquette University School of Medicine, Milwaukee*  
 The renal lesion of murine haemobartonellosis compared with lower nephron nephrosis
- 5 **J H Grindlay, E V Flock and J L Bollman**, *Division of Experimental Medicine, Mayo Foundation, Rochester*  
 Hepatic lymph and ascitic fluid following experimental chronic obstruction of the inferior vena cava
- 6 **James H Baxter** (introduced by Donald D Van Slyke), *Hospital of the Rockefeller Inst for Medical Research, New York*  
 Circulatory disturbances in hepatic and renal cortical necrosis
- 7 **Arthur A Nelson and Geoffrey Woodard** (by invitation), *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington*  
 Adrenal cortical atrophy and liver damage produced in dogs by feeding 2,2 BIS-(parachlorophenyl) - 1,1 - dichloroethane (DDD)
- 8 **Benjamin Highman**, *National Institute of Health, Pathology Laboratory, Washington*  
 Influence of various protective compounds in preventing fatty changes by dichloroethane
- 9 **Hans Popper, Frederick Steigmann, Alvin Dubin** (by invitation) and **Hattie M Dyniewicz** (by invitation), *Hektoen Institute for Medical Research of the Cook County Hospital, Chicago*  
 The influence of lipid ingestion on the thymol turbidity test
- 10 **Rolf Lium** (by invitation) and **Stephen Maddock**,

*Surgical Research Laboratory of the Boston City Hospital*

Experimental production of acute pancreatitis

- 11 M H F Friedman, B F Haskell (by invitation), and J M Waldron (by invitation) *Department of Physiology, Jefferson Medical College and Department of Surgery, Jefferson Hospital, Philadelphia*

Treatment of non specific ulcerative colitis for one year with intestinal extracts (Physiol)

### **PATHOLOGY B**

*Tuesday, March 16, 9 00 a m*

ROOM 16, CONVENTION HALL

#### **Immunology**

- 1 Edward C Rosenow, *Rare Metals Institute of the California Institute of Technology, Pasadena, and the Longview Hospital, Cincinnati*

Production *in vitro* from protein and other solutions of substances resembling "natural" agglutinins and precipitins

- 2 Richard H Follis, Jr *Department of Pathology, Johns Hopkins University*

Respiration of tissues from hypersensitive animals when antigen is added *in vitro*

- 3 L W Roth, R K Richards, and I M Shepperd (by invitation) *Department of Pharmacology, Abbott Laboratories, North Chicago*

Production of anaphylaxis in guinea pigs with weakly antigenic protein hydrolysates (Physiol)

- 4 Ernest Kun (Introduced by C Phillip Miller) *Department of Pharmacology and Medicine, University of Chicago, Chicago*

The effect of bacterial endotoxins on the carbohydrate metabolism of the rabbit

- 5 Walter D Hawk (by invitation) and C Phillip Miller, *Department of Medicine, The University of Chicago, Chicago*

Protection against bacterial endotoxin by penicillin and its impurities

- 6 A P Krueger, T Cohn (by invitation) and P N Smith (by invitation), *Department of Bacteriology and Office of Naval Research Task V, University of California, Berkeley*

The production of phage in the absence of cellular growth

- 7 Kjell Agner (introduced by A M Pappenheimer, Jr) *Chemical Laboratory of the Serafiner Hospital, Stockholm, and the Department of Bacteriology, New York University College of Medicine*

Detoxification of diphtheria toxin by peroxidase (Biochem)

- 8 Robert T Thompson and Frances E Moses (introduced by M A Blankenhorn), *Departments of Internal Medicine and Biochemistry, College of Medicine, University of Cincinnati*

Specificity of hum in serum antihyaluronidase for antagonism of a particular species of bacterial hyaluronidase

- 9 William E Ehrlich, Carolyn Forman (by invitation) and Joseph Seifter, *Philadelphia General Hospital, University of Pennsylvania, Wyeth Institute Applied Biochemistry*

A study of experimental serum sickness

### **JOINT SESSION OF THE FEDERATION**

*Tuesday, March 16, 1 30 p m*

BALLROOM, CONVENTION HALL

Program on page 311

### **PATHOLOGY BUSINESS MEETING**

*Tuesday, March 16, 4 15 p m*

ROOM 15, CONVENTION HALL

### **PATHOLOGY A**

*Wednesday, March 17, 9 00 a m*

ROOM 20, CONVENTION HALL

### **JOINT SESSION WITH THE AMERICAN PHYSIOLOGICAL SOCIETY**

#### **Coagulation**

- 1 R H K Foster, *Department of Pharmacology, St Louis University School of Medicine*

Coagulation in plain and partially heparinized plasma

- 2 Arnold G Ware (by invitation) and Walter H Seegers, *Department of Physiology, Wayne University, College of Medicine*

Serum accelerator globulin, quantitative determination, purification and properties

- 3 L M Tocantins, R T Carroll (by invitation) and Thomas McBride (by invitation), *Division of Hematology, Department of Medicine, Jefferson Medical College, Philadelphia*

Lipid inhibitors and accelerators of blood coagulation in extracts of human brain



- 4 Bernard G H Thomas (by invitation),  
Jacqueline Spears (by invitation) and Charles  
R Linegar, *Pharmacological Development  
Division, E R Squibb and Sons, New  
Brunswick*

Factors affecting the use of lung thromboplastin in the determination of prothrombin time

- 5 John H Ferguson (by invitation) and Jessica  
H Lewis, P W Boyles, B L Travis and  
E B Gerheim, *Department of Physiology,  
University of North Carolina, Chapel Hill*  
Studies on the stability, inhibition and activation of fibrinolytic protease

- 6 Walters H Seegers and Arnold G Ware (by  
invitation), *Department of Physiology, Wayne  
University, College of Medicine*

Purification of prothrombin and thrombin

- 7 F L Munro and Muriel Platt Munro (introduced by F R Miller), *Charlotte Drake  
Cardaza Foundation, Department of Medicine, Jefferson Medical College and Hospital, Philadelphia*

Purification of a substance from bovine serum which reduces the prothrombin time of aged plasma

- 8 Mario Stefanini (by invitation) and Armand J Quick, *Department of Biochemistry, Marquette University School of Medicine, Milwaukee*

The concentration of the labile factor of prothrombin in the blood of various species (Biochem)

- 9 Marjorie B Zucker, *Department of Physiology, College of Physicians and Surgeons, Columbia University*

The effect of thrombin injections on hemostasis

- 10 Armand J Quick, *Department of Biochemistry, Marquette University School of Medicine, Milwaukee*

Role of platelets in coagulation of blood, evidence of an inhibitor of the platelet factor (Biochem)

- 11 F D Mann, Margaret Hurn and D R Mathieson (introduced by T B Magath), *Division of Clinical Laboratories, Mayo Clinic, Rochester*

The platelets as foci in the coagulation of the blood

- 12 Alfred Lewin Copley, *Marine Biological Laboratory, Woods Hole, and Laboratory of Cellular Physiology, Department of Biology, New York University, New York*

Embolization of platelet agglutination thrombi in the hamster's pouch produced by heparin

## PATHOLOGY B

Wednesday, March 17, 9 00 a m

ROOM 16, CONVENTION HALL

### Vitamins, Hormones, Basement Membranes

- 1 Charlotte L Maddock (by invitation), S B Wolbach and Dorothy Jensen (by invitation), *Department of Pathology, Harvard Medical School and Surgical Research Laboratory, Boston City Hospital*

Hypoprothrombinemia with hemorrhage as a cause of death in the rat in hypervitaminosis A

- 2 Herbert C Stoerk (introduced by A M Pappenheimer, Sr), *Department of Bacteriology, Harvard Medical School, Boston*

Desoxypyridoxine morphologic and functional changes in acute pyridoxine deficiency

- 3 James F Rinehart and Louis D Greenberg (by invitation), *Division of Pathology, University of California, Medical School, San Francisco*

Arteriosclerotic lesions in pyridoxine deficient monkeys

- 4 Janet M Lemley, Robert G Gale, Robert H Furman, Mary E Charrington (by invitation) and George R Meneely, *Departments of Medicine and Biochemistry, Vanderbilt University School of Medicine*

Plasma tocopherol levels in cardiac patients

- 5 Hans Kaunitz and Charles A Slanetz (by invitation), *Departments of Pathology and Animal Care, College of Physicians and Surgeons, Columbia University, New York City*

Failure of implantation in vitamin E deficient rats

- 6 William Trager, *Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton*

Further studies on a fat soluble biotin active material (FSF) from plasma

- 7 Albert Segaloff and Richard L Coppedge (by invitation), *Departments of Medicine and Physiology, Tulane University and the Alton Ochsner Medical Foundation, New Orleans*  
Further studies on the hepatic potentiation of the estrogenic activity of triphenylchloroethylene

- 8 Isidore Gersh, *Department of Pathology, University of Illinois College of Medicine, Chicago*  
Histochemical studies of basement membranes

- 9 J F A McManus (introduced by R D Baker), *Department of Pathology, The Medical College of Alabama, Birmingham*

Histochemical features of the renal basement membrane

## PATHOLOGY A

Wednesday, March 17, 1 45 p m

ROOM 15, CONVENTION HALL

## Blood

- 1 J M Waldron (*by invitation*) and M H F Friedman, *Department of Physiology, Jefferson Medical College, Philadelphia*  
The relationship between anticoagulants and lipemia (Physiol)
- 2 E B Gerheim, (*by invitation*), J H Ferguson and B L Travis (*by invitation*), *Department of Physiology, University of North Carolina, Chapel Hill*  
Staphylocoagulase and staphylokinase (Physiol)
- 3 Virginia Minnich (*by invitation*) and Carl V Moore, *Department of Internal Medicine, Washington University School of Medicine, St Louis*  
Hypoplastic anemia induced in guinea pigs by 4 amino pteroyl glutamic acid
- 4 Reubenia Dubach (*by invitation*), Sheila Callender (*by invitation*) and Carl V Moore, *Department of Internal Medicine, Washington University School of Medicine, St Louis*  
Iron absorption in normal subjects and in patients with anemias of varied etiology
- 5 F S Robschert-Robbins, *Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester*  
The influence of folic acid on hemorrhage anemia in dogs
- 6 Valy Menkin, *Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia*  
The fate of aged leukocytosis-promoting factor of exudates
- 7 Harry Sobel (*by invitation*) and Jacob Furth, *Cornell University Medical College, New York City and Veterans Administration Hospital, Southwestern Medical College, Dallas*  
Experimental hypervolemia, time of onset and some associated physiological and chemical changes
- 8 Earl P Benditt, *Department of Pathology, The University of Chicago Clinics, Chicago*  
The relationship of caloric intake level and protein intake level to rate of protein synthesis
- 9 Roger Terry, (*introduced by G H Whipple*), *Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester*

Prolonged parenteral plasma produces hyperproteinemia and proteinuria in dogs and maintains nitrogen equilibrium and health

## PATHOLOGY B

Wednesday, March 17, 1 45 p m

ROOM 16, CONVENTION HALL

## Infection

- 1 Edmund P Finch (*by invitation*) and Seward E Owen, *Veterans Administration, Hines, Ill*  
Urinary tract infections and antibiotics (Physiol)
- 2 Clayton G Loosli, *Department of Medicine, University of Chicago, Chicago*  
The histogenesis of cells in pneumonia as seen in multiple lobe pneumococcus (type 1) infections in dogs
- 3 Joseph E Smadel and Elizabeth B Jackson (*by invitation*), *Department of Virus and Rickettsial Disease, Army Medical Department Research and Graduate School, Army Medical Center, Washington*  
Chemotherapeutic effect of chloromycetin on experimental infection with psittacosis and lymphogranuloma venereum viruses
- 4 F B Gordon, F M Schabel, Jr, (*by invitation*) and Margaret Abendroth (*by invitation*), *Department of Bacteriology and Parasitology, University of Chicago*  
Susceptibility of *macacus cynomolgus* to Japanese encephalitis virus with special reference to the alimentary canal
- 5 O M Gruhzit and R A Finken (*by invitation*), *Research Laboratories, Parke, Davis and Co, Detroit*  
Failure of trypanosome cruzi lysate in treatment of Brown-Pierce carcinoma of rabbits
- 6 Orlin Wood (*by invitation*), Doris Noshold (*by invitation*) and Lloyd D Seager, *Department of Pharmacology, Woman's Medical College of Pennsylvania*  
The effect of splenectomy upon the susceptibility of mice to infection by *trypanosoma cruzi* (Pharm)
- 7 Alfred Golden and R R Overman (*by invitation*), *Departments of Pathology and Physiology, University of Tennessee, College of Medicine*  
Sodium potassium levels and adrenal gland necrosis in experimental Simian malaria
- 8 R H Rigdon, *Department of Pathology, School of Medicine, University of Texas*  
Effect of blood and oxygen on P knowlesi infection in monkeys

- 9 Milton D Levine, Ray F Garzoli, Robert E Kuntz and John H Killough, (introduced by K M Endicott), *Naval Medical Research Institute, National Naval Medical Center*  
On the demonstration of hyaluronidase in cercariae of schistosoma mansoni

### Federation "Mixer"

Wednesday, March 17, 9 00 p m

ARENA, CONVENTION HALL

### PATHOLOGY A

Thursday, March 18, 9 00 a m

ROOM 15, CONVENTION HALL

### Cholesterol, Isotopes, Miscellaneous

- 1 Jesse L Bollman and Eunice V Flock, *Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota*  
Cholesterol in Intestinal and Hepatic Lymph of the Rat

- 2 Forrest E Kendall, Walter Meyer, and Margaret Bevans (introduced by H P Smith), *Research Service, First (Columbia) Division, Goldwater Mem Hospital, Dept of Hospitals, City of New York and the Dept of Med, College of Physicians and Surgeons, Columbia University*

Effect of intravenous injection of oxidized cholesterol upon the production of atherosclerosis in rabbits

- 3 Margaret Bevans, Liese Lewis Abell, and Forrest E Kendall (introduced by H P Smith), *From the Research Service, First (Columbia) Division, Goldwater Mem Hospital, Dept of Hospitals, City of New York, and the Dept of Med, College of Physicians and Surgeons, Columbia University*

Production of intimal atherosclerosis by intravenous injection of colloidal cholesterol into rabbits

- 4 Alfred Steiner, Margaret Bevans, and Forrest E Kendall, (introduced by H P Smith), *From the Research Service, First (Columbia) Division, Goldwater Mem Hospital, Dept of Hospitals City of New York, and the Dept of Medicine, College of Physicians and Surgeons, Columbia University*

Production of arteriosclerosis in dogs with cholesterol and thiouracil

- 5 Russell L Holman, *Dept of Pathology, L S U School of Medicine, New Orleans*  
Arterial disease may be a matter of days, not decades

- 6 P F Hahn, *Vanderbilt Univ School of Med, Nashville, Tenn*

Tumor therapy by the direct infiltration of radioactive colloidal metallic gold

- 7 George Rouser (introduced by P F Hahn), *Vanderbilt Univ School of Med, Nashville*  
Preparation of radioactive gold colloids for use in the therapy of malignancies

- 8 Herman N Eisen (by invitation) and Albert S Keston (by invitation), *Robert C Warner, Depts of Medicine and Chemistry, New York University, College of Med*

Immunochemical studies with proteins labelled with trace amounts of radioactive iodine (Biochem)

- 9 H G Davis, Jr (by invitation) and R D Baker, *Dept of Pathology, Medical College of Alabama*

Blood iodine in alloxan diabetes of dogs

- 10 Louis J Strobino (introduced by Lee E Farr) *Alfred I duPont Institute of the Nemours Foundation, Wilmington, Delaware*

Some factors associated with variations in nitrogen and ash content of bone

- 11 Frederick M Allen, *City Hospital, Welfare Island, New York, N Y*

Discrepant animal and clinical observations on hypothermia and procaine

- 12 Frank Dixon (by invitation) and Shields Warren, *Laboratory of Pathology, New England Deaconess Hospital, Boston*

Antigen tracer studies in anaphylactic shock

### PATHOLOGY

#### Motion Pictures

Thursday, March 18, 8 00 p m

ROOM C, CONVENTION HALL

Program on page 314

### Pathology

#### Papers Read by Title

- 1 Julius Schultz (by invitation) and Charles Weiss, *Laboratories of the Jewish Hospital, Philadelphia 41, Pa*

Hydrolysis of Di Leucylglycylglycine by sera of tuberculous and normal rabbits

- 2 Alfred Golden, *Department of Pathology, University of Tennessee, College of Medicine, Memphis, Tennessee*

Adrenal gland lesions in experimental simian malaria and similar human lesions in varied diseases

- 3 William Trager, *Dept of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton*

The inhibition of growth of lactobacillus casei by lyolecithin

## AMERICAN INSTITUTE OF NUTRITION

## TWELFTH ANNUAL MEETING

## NUTRITION

Tuesday, March 16, 9 00 a m

ROOM A, CONVENTION HALL

## Vitamins

- 1 Paul F Fenton (by invitation) and George R Cowgill, Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven

The pantothenate requirements of the mouse, with observations on the role of biotin, inositol and p-aminobenzoic acid

- 2 Elaine P Ralli, Mary E Dumm (by invitation), and Paul Roth (by invitation), Department of Medicine, New York University College of Medicine, New York

Pantothenic acid requirement of adrenalectomized rats and its relation to the white blood cells

- 3 Thomas R Riggs (by invitation) and D Mark Hegsted, Department of Nutrition, Harvard School of Public Health and Department of Biological Chemistry, Harvard Medical School, Boston

The effect of pantothenic acid deficiency on acetylation in rats

- 4 Walter C Russel and Arthur E Teeri (by invitation) (now at the University of New Hampshire, Durham), Department of Agricultural Experiment Station, Rutgers University, New Brunswick

Blood constituents of swine in a pantothenic acid deficient condition

- 5 J B Neilands (by invitation) and F M Strong, Department of Biochemistry, University of Wisconsin, Madison

The enzymatic liberation of pantothenic acid (Biochem )

- 6 W T Burnett (by invitation), R C Miller, and R A Dutcher, Pennsylvania State College, State College

Niacin and protein relationships in corn diets for growing pigs

- 7 W D Salmon, Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn
- The tryptophane requirement for growth of the rat

- 8 Herbert P Sarett and Grace A Goldsmith, Nutrition Research Laboratory, Tulane University School of Medicine, New Orleans

Effects of gelatin, glycine, and pyridoxine on tryptophane and nicotinic acid metabolism in humans

- 9 Ernestine I Frazier (by invitation), Thelma Porter, and Mary Jane Humphrey (by invitation), Department of Home Economics, University of Chicago

The utilization of nicotinic acid by pregnant women

- 10 Fred Rosen (by invitation), W A Perlzweig, and Philip Handler, Department of Biochemistry, Duke University School of Medicine, Durham

A fluorimetric assay for N<sup>1</sup> methyl 6 pyridone 3 carboxylamide (Biochem )

- 11 R W Engel (introduced by W D Salmon), Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn

Anemia in chronic choline deficiency in the rat

## Joint Session of the Federation

Tuesday, March 16, 1 30 p m

BALLROOM, CONVENTION HALL

Program on p 314

American Institute of Nutrition Dinner,  
Presentation of Awards, Business Meeting

Tuesday, March 16, 6 30 p m

## NUTRITION

Wednesday, March 17, 9 00 a m

ROOM A, CONVENTION HALL

## Vitamins

- 1 R M Johnson (by invitation) and C A Baumann, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

Relative importance of growth and metabolic rate on the utilization of vitamin A by the rat

- 2 Barbara Kelley (by invitation) and Harry G Day, Department of Chemistry, Indiana University, Bloomington

Effect of xanthophyll on the utilization of carotene and vitamin A by the rat

- 3 Jean Mayer (by invitation) and W A Krehl, Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven

Vitamin A deficiency and diet composition (Biochem )

- 4 Albert E Sobel, Lottie Besman (by invitation), and Benjamin Kramer, *Departments of Biochemistry and Pediatrics, The Jewish Hospital of Brooklyn, New York*  
Vitamin A absorption in newborns, older children, adults, and A storage in rats (Biochem)
- 5 E L Hove, *Research Laboratories, Distillation Products, Inc, Rochester, New York*  
 $\alpha$ -Tocopherol and certain nitrogenous compounds as factors influencing the mortality of rats after carbon tetrachloride poisoning
- 6 M L Quaife (introduced by P L Harris), *Research Laboratories, Distillation Products, Inc, Rochester, New York*  
Analytical procedures for determining individual tocopherols in a mixture of  $\alpha$ -,  $\beta$ ,  $\gamma$ , and  $\delta$  tocopherols (Biochem)
- 7 Mary B Mills (by invitation), Charlotte M Damron (by invitation), and Joseph H Roe, *Department of Biochemistry, George Washington University School of Medicine, Washington*  
Studies on the occurrence of diketol-L gulonic acid dehydro-L ascorbic acid and L ascorbic acid
- 8 Katherine J Elliott (by invitation), Shih Dzung Chen (by invitation), and Cecilia Schuck, *Department of Home Economics, Purdue University, Lafayette*  
Changes in dehydro and total ascorbic acid of cantaloupes on standing
- 9 J Walter Wilson and Elizabeth H Leduc (introduced by P H Mitchell), *Brown University, Providence*  
The effect of biotin on mitotic activity in the mouse liver (Biochem)
- 10 Frank Davis (by invitation) and George M. Briggs, *Department of Poultry Husbandry, University of Maryland, College Park*  
Studies on the cellulose growth factor for chicks
- 11 H R Bird, A C Groschke (by invitation), and Max Rubin (by invitation), *United States Department of Agriculture, Bureau of Animal Industry, Washington*  
Effects of arsenic acid derivatives in stimulating growth of chicks fed certain diets
- 2 Philip Handler and F Bernheim (by invitation), *Departments of Biochemistry and of Physiology and Pharmacology, Duke University School of Medicine, Durham*  
Dietary factors in experimental renal hypertension I Protein
- 3 A T Milhorat, *Departments of Psychiatry and Medicine, Cornell University Medical College, The Russel Sage Institute of Pathology and the New York Hospital, New York*  
Effect of arginine on urinary output of 17-ketosteroids in a patient with myotonia atrophica
- 4 George V Mann (by invitation) and Frederick J Stare, *Department of Nutrition, Harvard School of Public Health, and Department of Biological Chemistry, Harvard Medical School, Boston*  
Diet and dose response of weanling rats to intravenous alloxan
- 5 Julia O Holmes, L R Parkinson (by invitation), Anne W Wertz (by invitation), and Lois Brow (by invitation), *Agricultural Experiment Station, University of Massachusetts, Amherst*  
Dental caries in the rat, *Mus Norvegicus*
- 6 J Knox Smith (by invitation), E Potts Anderson (by invitation), Marie Zeppelin (by invitation), C A Elvehjem, and Paul H Phillips, *Department of Biochemistry, University of Wisconsin, Madison*  
Physical factors influencing dental caries in the cotton rat
- 7 Arild E Hansen, Oleeta Beck (by invitation), and Hilda Wiese (by invitation), *Department of Pediatrics, University of Texas Medical School, Galveston*  
Susceptibility to infection manifested by dogs on a low fat diet
- 8 Hilda F Wiese (by invitation) and Arild E Hansen, *Department of Pediatrics, University of Texas Medical School, Galveston*  
Lipid components of skin of dogs on low fat diet and dogs receiving lard
- 9 Robert P Geyer (by invitation), George V Mann (by invitation) and Frederick J Stare, *Department of Nutrition, Harvard School of Public Health and Department of Biological Chemistry, Harvard Medical School, Boston*  
The turbidimetric determination of infused fat in blood after intravenous administration of fat emulsions
- 10 C Boyd Shaffer and Frances H Critchfield (introduced by Paul L McLain), *Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh*  
The nutritive value of the fatty acids of lard esterified with a polyethylene glycol (Pharm)

## NUTRITION

Wednesday, March 17, 1 45 p m

ROOM A, CONVENTION HALL

### Nutrition and Disease, Lipids

- 1 Howard A Schneider, *The Rockefeller Institute for Medical Research, New York*  
The double strain phenomenon Biological basis of the nutritional effect in natural resistance to infection

- 11 **Harry H LeVeen** (*introduced by Frank Co Tur*),  
*Department of Surgery, New York University College of Medicine, New York*  
The use of natural and synthetic fat emulsions for intravenous feeding (Pharm)

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### Federation "Mixer"

Wednesday, March 17, 9 00 p m

ARENA, CONVENTION HALL

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### NUTRITION

Thursday, March 18, 9 00 a m

ROOM A, CONVENTION HALL

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#### General

- 1 **B L O'Dell** (*by invitation*) and **A G Hogan**  
*Department of Agricultural Chemistry, University of Missouri, Columbia*

Nutrients required by the rat for reproduction and lactation

- 2 **Sister Ann Miriam Allegeier, S C N** (*by invitation*), **Albert J Sica** (*by invitation*), **Leonora Mirone** (*by invitation*), **Frank P Panzarella** (*by invitation*), and **Leopold R Cerecedo**, *Department of Biochemistry, Fordham University, New York*

Studies on reproduction and lactation in rats and mice maintained on synthetic diets

- 3 **W A Krehl** and **I D Welt** (*by invitation*),  
*Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven*

Nutritional studies on the cat (Biochem)

- 4 **Pearl Swanson, W W Smith** (*by invitation*), **M Brush** (*by invitation*), and **H Merrian** (*by invitation*), *The Nutrition Laboratory of the Iowa Agricultural Experiment Station, Iowa State College, Ames*

Evaluation of adequate protein nutrition

- 5 **Mildred L Buckner** (*by invitation*), **Ruth Shively** (*by invitation*), and **Janice M Smith**, *Agricultural Experiment Station, Department of Home Economics, University of Illinois, Urbana*

Protein requirements of ten college women and adequacy of estimated quantities for ten weeks

- 6 **L R Richardson**, *Department of Biochemistry and Nutrition, A and M College of Texas, College Station*

Southern peas as a source of protein for growth

- 7 **Donald D Koroll** (*by invitation*), **William S Hoffman**, **Gordon McNeil** (*by invitation*), and **Hans Popper** (*by invitation*), *Holtzen Institute for Medical Research of the Cook County Hospital, Chicago*

The determination of the biological indices of gluten and gluten fortified with lysine in normal and protein deficient patients (Biochem)

- 8 **K A Kuiken** (*by invitation*), and **Carl M Lyman**, *Department of Biochemistry and Nutrition, A and M College of Texas, College Station*

The availability of amino acids in some foods (Biochem)

- 9 **Hazel E Munsell**, **Robert S Harris**, and **Louis O Williams** (*by invitation*), *Nutritional Biochemistry Laboratories, Massachusetts Institute of Technology, Cambridge*

Composition of Central American foods I Honduras

- 10 **Barnett Sure**, *University of Arkansas, Fayetteville*

A new dairy food

- 11 **A L Franklin** (*by invitation*), **J W Boehne III** (*by invitation*), and **T H Jukes**, *Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York*

Thiouracil content of poultry tissue following prolonged feeding of the compound

### NUTRITION

Thursday, March 18, 1 45 p m

ROOM A, CONVENTION HALL

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#### Mineral Nutrition

- 1 **Ruth Frenchman**, **Euphemia D Boroughs**, and **Frances A Johnston** (*introduced by Hazel M Hauck*), *New York State College of Home Economics, Cornell University, Ithaca*

The absorption of iron from beef by women

- 2 **Frances A Johnston**, **Euphemia D Boroughs**, and **Ruth Frenchman** (*introduced by Hazel M Hauck*), *New York State College of Home Economics, Cornell University, Ithaca*

The adequacy of an intake of seven milligrams of iron per day for women

- 3 **D Mark Hegsted**, **Clement A Finch** (*by invitation*), and **Thomas D Kinney** (*by invitation*), *Department of Nutrition, Harvard School of Public Health, the Departments of Biological Chemistry, Medicine and Pathology, Harvard Medical School,*

and the Medical Clinic and Department of Pathology, Peter Bent Brigham Hospital, Boston

The effect of diet upon iron absorption

- 4 Leon M Sharpe (by invitation), Robert S Harris, Wendell C Peacock (by invitation), and Richard C Cooke (by invitation), Departments of Food Technology and Physics, The Massachusetts Institute of Technology and the Walter E Fernald State School, Cambridge

Effect of phytate and other food ingredients on the absorption of radioactive iron

- 5 L B Pett and G F Ogilvie (by invitation), Nutrition Division, Department of National Health and Welfare, Ottawa, Canada

Hemoglobin levels by age and sex

- 6 Beula V McKey (by invitation), Eleanor Smith (by invitation), and Janice M Smith, Agricultural Experiment Station, Department of Home Economics, University of Illinois, Urbana

Calcium requirements of seven adolescent girls

- 7 Margaret G Morehouse (by invitation), Amber Lieng-shan Cheng (by invitation) and Harry J Devel, Jr, University of Southern California School of Medicine, Los Angeles

Studies on fat digestibility, the effect of melting points and dietary calcium and magnesium levels

- 8 H O Kunkel (by invitation) and P B Pearson, Department of Biochemistry and Nutrition, A and M College of Texas, College Station

The quantitative requirement of the rat for magnesium and effects of magnesium deficiency in the rabbit

- 9 Robert M Hill and Dorsey E Holtkamp (by invitation), Department of Biochemistry, University of Colorado Medical Center, Denver

Manganese and thiamine in the diet of mother rats and body temperature control in the young (Biochem)

- 10 Edith M Carlisle (by invitation) and Helen T Parsons, Department of Home Economics, University of Wisconsin, Madison

Achromotrichia produced in mice on a cooked egg diet

- 11 Esther DaCosta and Ruth Clayton (introduced by R E Johnson), U S Army Medical Nutrition Laboratory, 1849 W Pershing Road, Chicago

Changes in the water content of the albino rat during the first week of dietary rehabilitation

## NUTRITION BUSINESS MEETING

Thursday, March 18, 4 30 p m

ROOM A, CONVENTION HALL

## NUTRITION

Friday, March 19, 9 00 a m

ROOM A, CONVENTION HALL

## Vitamins

- 1 Grace A Goldsmith and Herbert P Sarett, Nutrition Research Laboratory, Tulane University School of Medicine, New Orleans

Urinary excretion of B vitamins in persons on normal and restricted diets

- 2 Wanda I Lameck (by invitation), Margaret N Coryell (by invitation), Eliot F Beach, and Icie G Macy, Research Laboratory, Children's Fund of Michigan, Detroit

Urinary thiamine and riboflavin excretion of children during fasting and under conditions of loading test

- 3 W O Caster (by invitation), Olaf Mickelsen, and Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis

Influence of dietary and physical factors on the urinary excretion of thiamine and pyrimin (Biochem)

- 4 Gladys Everson (by invitation), Eleanor Williams (by invitation), Elisabeth Wheeler (by invitation), Pearl Swanson, Margaret Eppright (by invitation), and Mattie Spivey (by invitation), The Nutrition Laboratory, Iowa Agricultural Experiment Station, Iowa State College, Ames

Occurrence of B vitamins in tissues of rats fed rations satisfactory and unsatisfactory for reproduction

- 5 H B Pierce, R F Krause (by invitation), J H Browe (by invitation), and Susan Merrow (by invitation), Departments of Biochemistry, Medicine, and Anatomy, College of Medicine University of Vermont, Burlington

The effect of vitamin therapy on serum blood levels

- 6 Harold P Morris, Celia S Dubnik (by invitation), and Thelma B Dunn (by invitation), National Cancer Institute, USPHS, Bethesda

Studies of thiamine deficiency in C3H mice

- 7 J R Haag and P H Weswig (by invitation), Oregon Agricultural Experiment Station, Corvallis

- 9 I N Dubin (introduced by A F Coca), *Division of Pathology and Bacteriology, University of Tennessee College of Medicine*  
Studies in hypersensitivity in simian malaria
- 10 Paul de Gara and Henry P Goldberg (by invitation), *New York Hospital and Dept of Pediatrics, Cornell University Medical College*  
Complement activity of sera from healthy and sick children
- 11 Christine E Rice, *Division of Animal Pathology, Science Service, Dominion Dept of Agriculture, Animal Diseases Research Institute, Hull, Quebec, Canada*  
Paralleled use of the "direct" and "indirect" complement-fixation tests
- 12 Manfred M Mayer and Charles C Croft (by invitation), *Dept of Bacteriology, Johns Hopkins School of Hygiene and Public Health*  
On the kinetics of hemolysis by antibody and complement

### IMMUNOLOGY

Wednesday, March 17, 1 15 p m

Room 10, CONVENTION HALL

- 1 Frank Maltaner and J O de Almeida (by invitation), *New York State Dept of Health, School of Medicine, University of Sao Paulo, Brazil*  
The effect of MG<sup>++</sup> on the complementary and coagulative activities of blood serum
- 2 Daniel Boroff, *Camp Detrick*  
A study on toxins and antigens of *S dysenteriae*
- 3 Edgar E Baker (by invitation), Ely Perlman and Walther F Goebel, *Rockefeller Institute for Medical Research and the Mt Sinai Hospital, New York*  
The isolation and properties of specific antigens from variants of *Shigella Sonnei*
- 4 E Singer, S H Wei and S H Hoa (introduced by A F Coca), *Central Bacteriological Laboratory, National Institute of Health, Nanking, China*  
Immunological studies of cholera filtrates
- 5 Melvin Cohn (by invitation) and A M Pappenheimer, Jr, *Dept of Bacteriology, New York University College of Medicine*  
Diphtheria toxin antitoxin reaction in human antisera
- 6 James A Harrison, Machteld E Sano (by invitation), Elizabeth H Fowler (by invitation), Robert H Shellhamer (by invitation), and Carol A Bocher (by invitation), *Dept*

*of Biology, Temple University, and Temple University School of Medicine and Hospital*  
Toxicity for paramacia of sera from cancerous and non cancerous persons

- 7 A Packchianian, *University of Texas School of Medicine*  
The production of anti-rabbit hemolysin (rabbit erythiolysin) in sheep, and its value for complement fixation tests
- 8 J McBroom Junge and S M Rosenthal (introduced by A F Coca), *Division of Physiology, National Institute of Health, Bethesda, Md*  
Effect of environmental temperature upon sulfadiazine therapy, body temperature and oxygen consumption in pneumococcus infection
- 9 Morris N Green (introduced by Stuart Mudd), *Dept of Bacteriology, University of Pennsylvania School of Medicine*  
Studies on the mechanism of action of furacin (5-nitro-2-furaldehyde semicarbazone)
- 10 M G Sevag and Joseph S Gots (by invitation), *Dept of Bacteriology, University of Pennsylvania School of Medicine*  
Studies on pneumococcal enzymes involved in resistance to drugs
- 11 M G Sevag and Edward Steers (by invitation), *Dept of Bacteriology, University of Pennsylvania School of Medicine*  
The relation of tryptophane utilization to the mechanism of resistance to sulfonamides

### IMMUNOLOGY

Wednesday, March 17, 7 00 p m

Room 21, CONVENTION HALL

### Symposia

#### I SITE OF ANTIBODY FORMATION

- 1 Philip D McMaster, LEADER (by invitation), *Rockefeller Institute for Medical Research, New York*
- 2 Abraham White (by invitation), *Yale University*
- 3 Dan H Campbell, *California Institute of Technology, Pasadena*
- II ELECTRON MICROGRAPHY OF THE STRUCTURE OF BACTERIA AND VIRUSES
- 1 Ralph W G Wyckoff, LEADER, *National Institute of Health, Bethesda, Md*
- 2 Stuart Mudd, *University of Pennsylvania School of Medicine*
- 3 Geoffrey Rake, *Squibb Institute for Medical Research, New Brunswick*
- 4 Albert Claude (by invitation), *Rockefeller Institute for Medical Research, New York*



## Federation "Mixer"

Wednesday, March 17, 9 00 p m

ARENA, CONVENTION HALL

## IMMUNOLOGY

Thursday, March 18, 9 00 a m

ROOM 10, CONVENTION HALL

- 1 Elvin A Kabat, Murray Glusman (by invitation) and Vesta Knaub (by invitation), Depts of Neurology and Bacteriology, College of Physicians and Surgeons, Columbia University, and the Neurological Institute of New York

Immunochemical estimation of albumin and gamma globulin in normal and pathological cerebrospinal fluid

- 2 David Pressman and Geoffrey Keighley (introduced by A F Coca), Gates and Crellin Laboratories of Chemistry and the William G Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

The zone of activity of antibodies as determined by the use of radioactive tracers

- 3 Herman N Eisen and Albert S Keston (introduced by Robert C Warner), Depts of Medicine and Chemistry, New York University College of Medicine

Measurement and study of immune precipitates by means of ultraviolet absorption spectroscopy (Biochem)

- 4 Ellis Bolton, Charles Leone and Alan Boyden (introduced by A F Coca), Bureau of Biological Research and Dept of Zoology, Rutgers University

A critical analysis of the performance of the photoreflexometer in the measurement of serological and other turbid systems

- 5 Henry P Treffers and Katherine E Yaw (by invitation), Section of Immunochemistry, Dept of Bacteriology and Immunology, Yale University School of Medicine

A turbidimetric growth assay method for the determination of the relative bactericidal activities of sera

- 6 John P Fox (introduced by Peter K Olitsky), Laboratories of the International Health Division, Rockefeller Foundation, New York

The long persistence of *Rickettsiae orientalis* in the blood and tissues of infected animals

- 7 Edward C Rosenow, Rare Metals Institute of the California Institute of Technology, Pasadena, and Longview Hospital, Cincinnati

Further studies on the production *in vitro* from bacteria of substances resembling "natural" agglutinins and precipitins

- 8 William Burrows, Dept of Bacteriology and Parasitology, University of Chicago

Excretion of antibody in feces and urine and its absorption from the bowel

## IMMUNOLOGY

## Papers Read by Title

- 1 I N Dubin, J D Reese (by invitation) and Lois A Seamans (by invitation), Division of Pathology and Bacteriology, University of Tennessee College of Medicine, and Dept of Health and Safety, Tennessee Valley Authority

Attempt to produce protection against mosquitoes by active immunization

- 2 John P Fox and Osler L Peterson (introduced by Peter K Olitsky), Laboratories of the International Health Division, Rockefeller Foundation, New York

Mode of action of thionine dyes in combatting experimental rickettsial infections of mice

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# Federation Proceedings

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## NOTES ON THE ATLANTIC CITY CONVENTION OF THE FEDERATION

MARCH 15 TO 19, 1948

The thirty-second annual meeting of the Federation was held at Atlantic City, New Jersey, March 15 to 19, 1948. The Haddon Hall Hotel was the headquarters hotel for the meeting and meetings of the Councils of the constituent Societies and of the Federation Executive Committee were held there, beginning Sunday, March 14. The Municipal Auditorium of Atlantic City was the locus of all of the scientific, general and symposium sessions, of the commercial exhibits and of the social function held in the evening of March 17.

Dr. Maurice H. Seevers, President of the American Society for Pharmacology and Experimental Therapeutics, was Chairman of the meeting. Ninety-six scientific sessions were held, beginning Tuesday, March 16 at 1:00 p.m. and ending Friday afternoon. The Joint Session of the Federation was held on Tuesday afternoon, and symposia were presented by the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics and the American Association of Immunologists. A total of 1122 papers appeared in the program.

Registration was 3324, divided about equally between Society members and non-members.

The exhibits of commercial firms, held for the first time at the Federation meeting, were of a high order of quality and were well received and approved by members and exhibitors alike. It is planned to continue this as an important feature of future meetings. There was no opportunity at this meeting for scientific demonstrations by members. It is hoped that in the future it will be possible to have a number of scientific exhibits to partially compensate for the lack of demonstrations.

Actions taken by the Federation Executive Committee that are of general interest were the following:

1 Decision to hold the 1949 meeting in Detroit, Michigan, in the week of April 18.

2 Decision to hold the meeting in 1950 in Atlantic City, probably in April. The 1951 meeting is tentatively scheduled to be held in San Francisco, California.

3 The Federation assessment for 1948 was set at \$3.00 per member of each constituent Society. This includes subscription to *FEDERATION PROCEEDINGS*.

4 Dr. M. O. Lee was appointed Federation Secretary-Treasurer and Managing Editor of *FEDERATION PROCEEDINGS*, with offices at 2101 Constitution Ave., Washington 25, D. C.

5 Approval of recommendations of the Control Committee to: a) accept advertising for *FEDERATION PROCEEDINGS*, and b) to allow authors of full-length articles in the Proceedings approximately one page of illustrations without charge, any excess to be charged to the author at the discretion of the Control Committee. These recommendations were made to avoid a deficit in financing the Proceedings.

6 Approval of recommendations of the Secretaries' Committee on Program Reorganization to:

a Hold the Joint Session in the evening at the 1949 meeting, with the American Society for Experimental Pathology to be responsible for arranging the program.

b Limit the number of simultaneous scientific sessions at the annual meeting to a minimum of 12 over a period of four days.

c Permit any Society to schedule regular scientific sessions on the Federation program on one or more evenings.

d Hold the Annual Meeting each year in the third or fourth week of April whenever possible.

7 Dr. M. O. Lee and Dr. William H. Chambers were delegated to prepare and submit a revision of the By-laws of the Federation to the Executive Committee for consideration.

## CORRECTIONS OF ABSTRACTS IN MARCH ISSUE

**Page 25 DANOWSKI** Line 8 should read "The addition of  $\text{NiCl}$  or of  $\text{KCl}$ ", line 9, delete the word "its", line 11, delete " $\text{KCl}$  or"

**Page 111 SHANES** Line 11, delete last word, "to", line 13, the words "but increases" should read "and perhaps"

**Page 112 SHANES** Line 1, delete comma after the word "prolonged" **SHAPIRO** Line 11, the equation  $(P(V - b) \% K)$  should read  $(P(V - b) = K)$

**Page 132 WESSON, ANSLOW, AND SMITH** The first author's name should be followed by "Jr."

**Page 154 FERRY AND SHULMAN** Line 9, the word, "synergizing" should read "synerizing"

**Page 160 HIRSCHMAN AND HIRSCHMAN** Column 2, line 7, "20 acetonyllopregninedione-3, 16" should read "20 acetonyllopregninedione-3, 16", column 2, line 9, "-25°" should read "+25°"

**Page 168 LEVINE AND MERLIS** Column 1, line 6 from the bottom, the word "benzothio-  
phene" should read "dibenzothio-  
phene", column 2, last line, should read "a lower deep green ring  
with formaldehyde"

**Page 176 NEURATH AND ELKINS** Column 2, line 22, the sentence beginning "This conclusion" should read "The inhibition by D-phenyl-  
aluminum has been found to be of the competitive  
type"

**Page 178 PEARLMAN** Line 20, the words "a  
diacetyl" should read "in acetyl"

**Page 256 SMITH, GIMBLE AND DAVISON** Line 3, the sentence beginning "From the Department" should read "From the Toxicology Section  
of the Army Medical Department, Research and  
Graduate School and the Department of Pharma-  
cology, The George Washington University  
School of Medicine, Washington, D. C."



## Joint Session of the Federation

Atlantic City, March 16, 1948

MAURICE H. SEEVERS, *Chairman*

### THE PROBLEM OF TISSUE PROTEIN SYNTHESIS

PAUL R. CANNON

*Department of Pathology, The University of Chicago*

The widest gaps in our understanding of the mechanisms of protein metabolism are in relation to its anabolic phases. Indeed, with reference to the subject as a whole, one might say that we are still largely in the 'catabolic phase'. Although dietary proteins, after digestion and absorption, are presumably rebuilt by intracellular kathepsins into characteristic and specific proteins of various kinds, not much is yet known about the site or sites of the synthesis, i.e., whether it is primary in the liver and secondary elsewhere, or whether it proceeds concurrently in many tissues. Neither is a great deal known about the mechanisms which so unerringly assemble the building blocks of protein, whether as amino acids, peptides or protein aggregates, into specific tissue proteins. In the present discussion it will be assumed that in this assemblage certain mechanisms, especially the hypophysis and the genes, direct the synthesis but that this direction may be modified by various circumstances, as, for example, by the availability of dietary constructive units and by the influence of energy-yielding reactions and integrative mechanisms, particularly enzymes, salts and vitamins.

For the study of the complicated problems of tissue synthesis a variety of experimental procedures is needed, and the urgent need for a direct chemical approach is obvious. Unfortunately, however, the latter approach is difficult because of the unavoidable artificialities inherent in the use of excised tissues, with the deprivation of the blood supply and the ensuing proteolysis. It would seem important therefore, wherever possible to study the problem in intact animals whose physiologic mechanisms are not irreversibly damaged. Such an approach, while admittedly peripheral to the core of the broad problem of intermediary metabolism, nevertheless has been extremely useful in the past and still offers many advantages in relation to the overall problem.

Our interest in the subject of tissue protein synthesis arose in the course of studies of problems of convalescence in which we observed the effects of protein repletion in protein-depleted animals (1). The following collaborators participated actively in experiments upon which the discussion is based: Earl Benditt, William Chase, Laurence Frazier, Eleanor Humphreys, Donald Rowley, Harold Steffee, Robert Stepto, Robert Woolridge and Robert Wissler. As an experimental animal we have used the adult male albino rat made protein-deficient by a low protein diet (2). In from two and one-half to three months on such a diet the animals gradually lose weight and become markedly hypoproteinemic and moderately anemic, despite an adequate intake of calories, vitamins and salts. Under proper circumstances, however, such animals can resynthesize different kinds of bodily protein remarkably well, thereby demonstrating that any harmful effects induced by the low protein diet are readily reversible. We call this method the Rat-Repletion Method, by it we can measure rapidly, i.e., within from 7 to 14 days, the capacity of the animals to regain lost weight and to regenerate various types of tissue protein, as, for example, striated muscle, plasma protein, antibody protein, hemoglobin, liver protein, enzymes and carcass protein.

Our standard repletion ration, when fed in daily portions of 15 grams, furnishes 1.35 grams of high quality protein, 48 calories, and the vitamins and salts considered necessary for adequate nutrition of the rat. In seven days a rat eating all of this ration each day will regain from 35 to 40 grams of lost weight, in 10 days, from 50 to 55 grams, and in 14 days, from 65 to 70 grams. Simultaneously he will also regenerate the other types of tissue proteins already mentioned. Moreover, if a mixture of 16 crystalline or purified amino acids, compounded in the proportions as natural forms in which these occur in casein, is

substituted for the protein in the basal ration, the animal in 10 days will regain from 40 to 50 grams of lost weight, thus demonstrating that the amino acid mixture can replace protein quite effectively (3). Even though such a mixture may not be a complete substitute for natural protein, it nevertheless offers a useful means for the study of many phases of amino acid utilization.

The following experiments illustrate briefly a few features of the method. For example, if isocaloric rations containing proteins at approximately similar nitrogen concentrations are fed to groups of protein-depleted rats, it is possible within seven days to differentiate high-quality, intermediate-quality and low-quality proteins, whether in terms of weight recovery or of increase in concentration of total serum protein. Moreover, if a poor-quality protein, such as that present in white flour, is tested, with and without amino acid supplementation, one can demonstrate in 14 days the limiting effect of lysine in the flour and the elimination of this effect by appropriate supplementation. In fact, a foodstuff in which lysine has been injured or made unavailable for assimilation because of the injurious action of a high temperature can be restored practically to its original nutritive value by lysine supplementation (4). The method can be used also to ascertain the amino acid completeness of cancer protein. Thus when cancer metastases from the liver were compared with noninvolved portions of liver from the same patient, both types of tissue having been added to the basal ration at similar nitrogen concentrations, it was found that the cancer protein acted as a high-quality protein, and, therefore, contains an adequate content of all the essential amino acids. When three groups of rats are injected simultaneously with antigen it is possible to demonstrate the decreased capacity of severely depleted animals to fabricate specific antibody protein, in comparison with well-nourished rats, and that a week's repletion with high-quality protein enables depleted animals to recover much of their capacity to form antibody (2). It should be emphasized, however, that in the production of protein deficiency, both time, and extent of the depletion are essential factors, just as they are in relation to any other nutritional deficiency, whether it be of vitamins, calories or salts. If groups of protein-depleted rats are fed isocaloric rations containing similar protein concentrations, one protein (lactalbumin-casein), however, being high-quality, and the other (wheat gluten) low-quality, and with the rations pur-fed to the level

of consumption of the ration containing the low-quality protein, one can demonstrate, whether in terms of weight recovery, regeneration of total circulating plasma protein, total circulating hemoglobin, total carcass protein, or total liver protein, that there is no marked difference in relative repletion performances. For practical purposes this demonstrates that determination of total weight recovery is a reliable measure of the nutritive value of the protein being tested. Moreover, in each category it is apparent that calories do not compensate for the amino acid incompleteness of the wheat gluten. Finally, it can be shown by this method that amino acid mixtures, whether they consist of 16 amino acids, or only the 10 essential amino acids, can substitute quite effectively for natural protein in the basal ration. With such an experimental model it is possible, therefore, to study various aspects of protein metabolism under selective conditions.

In most of our work we have been concerned mainly with the structural aspects of tissue synthesis, particularly in relation to the utilization of indispensable amino acids. To a lesser degree we have been interested also in the energetics of the syntheses. It is obvious, however, that all phases of the process of synthesis are necessarily interrelated.

The classic experiments of Osborne and Mendel (5) emphasized one basic principle in particular, viz., that in the processes of protein synthesis the nutritive efficiency of a dietary protein is determined by the smallest amount within it of a single indispensable amino acid. From this arose the idea of essential amino acids as limiting factors for growth. Later, Rose and his associates (6) demonstrated the existence in the mammal of 8 or 10 of these essential amino acids, depending upon the species. In other words the mammal's 'synthetic disability', as Baldwin terms it (7), pertains to approximately 40 per cent of the known dietary amino acids. The more recent demonstrations by Rose and his collaborators (8) that the dietary absence in healthy men of any one of eight indispensable amino acids leads to a marked loss of body nitrogen, appetite and the sense of well-being, makes it evident that even for the maintenance of the presumably minimal degree of protein synthesis necessary for the 'continuing metabolism' postulated by Borsook and Keighley (9), at least all eight indispensable amino acids must be available to the synthesizing mechanisms.

In the experiments just cited the metabolic

mechanisms have had access to an adequate supply of other dietary essentials, viz., calories, vitamins and salts. There are instances, however, particularly in conditions of altered nutrition, in which these other dietary constituents are not available to the synthesizing mechanisms, and it is in this area especially that one may hope to elucidate further some of the complex mechanisms of protein synthesis.

In preparation for an experimental study along these lines one might ask the following questions:

1. How much energy is required by the synthesizing mechanisms in order to ensure the formation of the peptide linkages concerned in protein synthesis?

2. Above this level, will a higher caloric intake improve the efficiency of the synthesis?

3. Do vitamins function in the processes of synthesis, and if so, in what ways?

4. Are 'stores' or 'reserves' of essential amino acids available in the tissues for emergency function comparable to the reserves of glycogen, fat and protein?

5. Is the process of synthesis slow and intermittent or is it immediate and chain-like?

6. Are all essential amino acids simultaneously required for synthesis of a complete tissue protein?

7. Is there a daily rate of utilization of essential amino acids, both individually and totally, and is this modified by variations in physiologic and pathologic states?

Answers to some of these questions are already known, for others answers are still lacking. At least one thing is certain, viz., that under conditions of inadequate caloric intake the mammalian body tends to consume its available stores of carbohydrate, fat and protein. Obviously therefore, if tissue proteins are to be conserved for their specific functions, caloric maintenance requirements must be satisfied.

The relative influence of caloric and protein intake upon tissue synthesis was demonstrated by us in experiments (10) in which groups of protein-depleted rats were fed rations for 14 days in which *a*) protein consumption was kept constant and the caloric intake varied, and *b*) in which the caloric intake was kept at a constant high level (48 calories per rat per day) and the protein intake varied. In the first experiment weight recovery was retarded at intakes of 15 and 25 calories per rat per day whereas with intakes of 35, 48 and 60 calories there was comparatively little difference in weight gains. Moreover, at the

latter two levels carcass analyses showed that much of the added weight was due to deposition of fat rather than to synthesis of tissue protein. In the second experiment, on the other hand, with identical caloric intakes in all groups, tissue protein synthesis, as manifested by weight recovery, varied directly with protein consumption, except at the higher levels of protein intake where maximal capacity of utilization had been exceeded. Finally, when groups of depleted rats were fed breakfast foods at equal caloric intakes, enriched with additional vitamins and minerals, but with some of the foods having been damaged by processing procedures, weight recovery was correlated, not with caloric intake, for this was always the same, but with protein quality.

From these experiments we conclude *a*) that, below a certain level, caloric intake determines the efficiency of protein synthesis, whereas above this level additional calories act mainly to help lay down additional fat, and *b*) with an adequate caloric intake, the efficiency of protein synthesis depends directly upon protein quality and quantity. From the practical viewpoint, these findings would indicate that, above caloric maintenance levels, calories are of nutritional significance more with respect to energy reactions than in relation to protein synthesis.

If the processes of protein synthesis are dependent upon the combination of an adequate supply of building units of protein and of caloric energy, how is this energy made available to the synthesizing mechanisms? Undoubtedly the energy transfer is intermediated by enzyme systems and vitamins, but comparatively little is known as to the precise rôle of individual vitamins in the processes of synthesis. We have attempted, therefore, to ascertain what part the vitamins of the B complex may play by removing them, all at once or singly, from the repletion ration, in order to observe the consequences of their absence. Although these experiments are still incomplete and will be reported upon later in detail, it is evident that when a ration whose dietary nitrogen is supplied by a mixture of 16 crystalline amino acids is fed with all B complex vitamins absent, protein synthesis, as manifested by weight recovery, comes to a standstill within 10 days. Moreover, when only one vitamin at a time is removed from the ration, there is considerable variation in the response of the repleting rats, but with the absence of riboflavin manifesting the most conspicuous effect.

Assuming then that for the aggregation of

amino acids into tissue proteins both sufficient supplies of energy and of vitamins are necessary, how are the amino acids themselves assembled by the synthesizing mechanisms into specific proteins? It is known, of course, that amino acids circulate constantly in the blood and should, therefore, be available for various tissue syntheses, and yet experiments have shown that, whether in a normal or a protein-depleted animal, a deficiency of only a few milligrams per day of a particular essential amino acid hampers effective protein synthesis, despite the adequate intake of all the others. Furthermore, it has been shown that under these circumstances there is an augmented excretion of amino acids in the urine. Evidently then the missing or deficient essential amino acid cannot be secured either from the circulating blood or by the 'raiding' of tissues, and in consequence catabolism is accelerated. This suggests, moreover, that there are no available 'stores' or 'reserves' of individual essential amino acids in the sense that we are accustomed to think of the protein reserves, defined by Madden and Whipple (11) as "all protein which may be given up by an organ or tissue under uniform conditions without interfering with organ or body functions."

Because of this apparent inability of the rat immediately to mobilize a particular essential amino acid for use by the synthesizing mechanisms in order to make up a particular essential amino acid deficit, it is possible to establish the minimal daily amount of each essential amino acid necessary, in relation to the others, for effective tissue protein synthesis. This can be done in the normal rat in terms of nitrogen equilibrium and maintenance of weight, and in the protein-depleted rat in terms of convalescence, as measured by weight recovery in a given time.

Such observations indicate that in the course of ordinary metabolism there is a daily 'turn-over' of each essential amino acid which necessitates a daily dietary replacement. In other words, for maintenance alone the essential amino acids are dietary expendables. Because of this fact it is possible to establish the daily utilization rate, or, as it is usually termed, the daily 'requirement' for each essential amino acid. These requirements differ, however, from time to time, depending upon circumstances which may accelerate expendability rates. Thus in an animal which has been severely depleted of tissue proteins there is a markedly augmented utilization of essential

amino acids during the repletion period, or, in other words, the animal's 'requirements' are markedly elevated. For example, in adult rats of comparable ages fed similar repletion rations, the utilization of essential amino acids may be increased from two fold to five-fold in protein-depleted animals in relation to the comparable utilization by normal rats. Moreover, this utilization rate is not uniformly elevated with respect to each amino acid. Thus in the severely depleted animals the utilization rate for lysine and leucine may be especially elevated. The question is: Why should there be this unequal utilization of these two essential amino acids in relation to the others?

An answer to this question may be found from a consideration of the amino acid composition of normal striated muscle. For example, recent analyses (Beach *et al.* (12), Greenwood and Kraybill) have shown that, among the essential amino acids of beef muscle, there is a proportionality relationship of approximately 8 to 1 between lysine and tryptophane and between leucine and tryptophane. In other words, in the resynthesis of muscle protein the repleting muscle presumably requires at least eight times as much lysine and leucine as it does tryptophane if the original proportionality relationships of these essential amino acids are to be maintained or reestablished. Since approximately one-third of the mammalian body consists of striated muscle, and inasmuch as in the course of protein depletion there is a marked loss of muscle mass in the processes of muscle atrophy, it is obvious that as a result of severe protein depletion there is a large tissue mass available to utilize dietary essential amino acids, provided that these also are available in proper proportions for effective utilization. If, on the other hand, the repletion diet provides an inadequate supply of lysine and leucine, the law of minimum presumably will come into play to restrict effective resynthesis of muscle protein, regardless of the diet's richness in other food constituents, whether these be calories, vitamins or minerals.

These observations re-emphasize the point of view that in the repletion of depleted tissues the dietary allowances of essential amino acids as well as those of other dietary constituents should be greater than those for maintenance, and that calculations based on maintenance figures should be modified to the extent that in the planning of a rehabilitation diet, maintenance allowances

should be increased proportionately. For example, if in a 3000 calorie diet the minimal protein allowance for maintenance is taken as 35 grams per day, it would seem advisable to increase this from two fold to five fold in order to supply a rich assortment of essential amino acids, and in proper proportions, to the synthesizing mechanisms.

How do the synthesizing mechanisms select amino acids from the bloodstream for assembly into tissue proteins? Does the synthesis proceed at a leisurely pace and intermittently, or is it a rapid process, resembling more a chain reaction, and in conformity with the ideas of Schoenheimer (13) and his associates, Borsook *et al.*, Whipple and Madden *et al.*, of the dynamic nature of protein metabolism? Accumulating evidence points more and more to the latter point of view and suggests that the synthesizing process is essentially an all or none type of mechanism. That is, if a complete tissue protein is to be synthesized, all the essential amino acids which are to enter into the process must be simultaneously available and in adequate amounts and proportions one to another during the act of synthesis. In other words, unless a tissue protein can be synthesized at once and completely, it may not be synthesized at all. This idea suggests that the synthesizing mechanism is 'perfectionistic' and will not utilize an inadequate assortment of amino acids. What evidence is there to support such a postulate?

It is not a new idea that in the utilization of essential amino acids for tissue synthesis there is a need for the simultaneous presence of several if not all of them, whether for growth or maintenance. As Rose has said (14) "if a tissue is to be formed at all, every component required must be available or be capable of being manufactured by the cells, otherwise the synthesis will not occur," and Burroughs, Burroughs and Mitchell (15) have concluded that even in the endogenous metabolism the utilization of some amino acids "is dependent upon the simultaneous presence of other amino acids in the metabolic mixture."

Evidence supporting these concepts may be cited briefly as follows.

1. Berg and Rose (16) observed in young rats fed a tryptophane deficient ration, that growth was better when tryptophane supplementation was made in divided doses rather than all at one time. Their interpretation of this phenomenon was that when tryptophane was fed singly in excess of the ability of the synthesizing tissues immediately to utilize it, the excess tryptophane

was wasted rather than being stored for later use when food again was eaten.

2. Elman (17) found that an acid hydrolysate of casein, supplemented with tryptophane, was well utilized when injected intravenously, whereas, if the hydrolysate was injected, followed six hours later by injection of the tryptophane, there was poor utilization of the hydrolysate, due presumably to the fact that the tryptophane could no longer combine with the other amino acids to accomplish tissue protein synthesis.

3. Geiger (18) fed young rats rations deficient in either tryptophane, methionine or lysine. Twelve hours later, when the missing amino acids were fed, effective growth did not occur. Here, too, the amino acids supplied by the deficient diets were apparently not stored within the tissues long enough to be available later for purposes of tissue synthesis when the missing essential amino acids were again made available.

4. Last year we reported experiments (19) in which protein depleted adult rats were fed repletion rations adequate in calories, vitamins and minerals but containing only ten essential amino acids as the principal source of dietary nitrogen. On such rations the animals quickly regained lost weight. If, however, the basal ration was divided into two portions, to one of which was added an amino acid mixture composed of arginine, histidine, leucine, lysine and threonine, and to the other a mixture composed of isoleucine, methionine, phenylalanine, tryptophane and valine, and these two incomplete rations were fed alternately at hourly periods with an hourly fasting period between each feeding, the animals continued to lose weight. When, however, the two incomplete rations were combined and fed under similar conditions, weight recovery was immediate. These experiments suggest, therefore, that for effective tissue synthesis all essential amino acids must be available in the tissues practically simultaneously, otherwise the first group absorbed is not stored long enough to enable its essential amino acids to combine with those of the second group for the synthesis of complete tissue proteins.

In this discussion an attempt has been made to emphasize dietary interrelationships in the processes of tissue protein synthesis, both with reference to the general interplay between indispensable amino acids, vitamins and calories and to the specific interrelationships of amino acids

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In this discussion an attempt has been made to emphasize dietary interrelationships in the processes of tissue protein synthesis, both with reference to the general interplay between indispensable amino acids, vitamins and calories and to the specific interrelationships of amino acids

as affected by differing proportionality patterns and limiting factors. Undoubtedly many other influences must be evaluated, as, for example, genes, hormones and minerals. In last analysis

the complete picture of tissue protein synthesis will probably resemble more a mosaic of all these elements than a series of independent and unrelated figures.

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# PHARMACOLOGICAL AND PHYSIOLOGICAL ASPECTS OF ADRENERGIC BLOCKADE, WITH SPECIAL REFERENCE TO DIBENAMINE<sup>1</sup>

MARK NICKERSON AND LOUIS S GOODMAN

*From the Department of Pharmacology, University of Utah School of Medicine*

In 1895, Oliver and Schrier (47) first observed the characteristic hypertension which follows the injection of extracts of the adrenal medulla. Today, after five decades, the abrupt and marked rise in blood pressure resulting from intravenously injected epinephrine is still one of the dramatic sights in experimental pharmacology.

But even more dramatic is the reversal of epinephrine-induced hypertension, which can be brought about by the prior administration of 'adrenolytic' chemicals. The phenomenon of *epinephrine-reversal* was first adequately described by Dale in 1906 (13) as an action of certain ergot alkaloids and has ever since attracted the attention of pharmacologists and physiologists. Indeed, it is difficult to find a pharmacologist who has not been occupied, at one time or another, with the fascinating problems of the mechanism and site of action of drugs producing adrenergic blockade, the physiological mechanisms disclosed by their administration, and their therapeutic potentialities.

## CLASSIFICATION OF AUTONOMIC BLOCKING DRUGS

Drugs causing adrenergic blockade are presented in table 1 which classifies autonomic blocking agents as a group. This table is based on two accepted facts. First, autonomic drugs act not on nerve endings, but on effector cells innervated by cholinergic or adrenergic nerves. Secondly, autonomic blocking agents selectively prevent the responses of effector cells to specific chemical mediators, either acetylcholine or sympathin, but they do not prevent responses to unrelated chemical substances or to direct stimulation.

Cholinergic innervation is either preganglionic or postganglionic but all adrenergic nerves are postganglionic. The specific effector cells innervated by each class of nerves are listed at the

bottom of the chart. For many and impelling reasons (12, 18) skeletal muscle is classified along with autonomic ganglia as an effector organ capable of responding to autonomic drugs.

The right-hand column of the table includes the various drugs which block the responses of smooth muscle, cardiac muscle and exocrine glands to adrenergic nerve impulses mediated by sympathin and to the hormone of the adrenal medulla, epinephrine.

**Terminology.** The term 'sympatholytic drug' has been reserved by some investigators for those agents which prevent effector cells from responding to adrenergic nerve impulses; the term 'adrenolytic drug' then applies to those agents which prevent responses to epinephrine. Usually, the two actions coexist but some chemicals have much greater adrenolytic than sympatholytic potency. A better designation applying to the group as a whole is 'adrenergic blocking agents'.

**Differences from tetraethylammonium.** The difference in site of action between adrenergic blocking drugs, such as Dibenamine and tetraethylammonium, an agent which has recently attracted considerable attention (3, 4, 28), deserves comment. Tetraethylammonium produces blockade of both sympathetic and parasympathetic ganglia. By paralyzing sympathetic ganglia, for example, it produces effects which are superficially similar to those observed after Dibenamine. However, tetraethylammonium, in conventional doses, does not act peripherally on muscle or exocrine glands, both remain sensitive to epinephrine and to postganglionic nerve impulses. Obviously, the site and the physiological consequences of the action of tetraethylammonium are quite different from those of Dibenamine.

**Properties of an ideal adrenergic blocking agent.** An ideal adrenergic blocking agent should have the following attributes. It should produce complete blockade, show a high degree of selectivity, possess a wide margin of safety, and have a fairly long duration of action. None of the hitherto available compounds possesses all these properties. In effective doses, each produces effects other than adrenergic blockade, most are rather feeble in potency, many are quite toxic, and some are

<sup>1</sup> Trademark of Givaudan-Delawanna, Inc. Figs. 2-7 and 9, courtesy of the *Journal of Pharmacology and Experimental Therapeutics* (40).

Fig. 12, courtesy of *The American Journal of Medicine* (22).

rather fleeting in action. For example, ergotamine causes not only adrenergic blockade but also stimulates the smooth muscle of blood vessels, the uterus and other organs, in addition, it has complex actions on the central nervous system (13, 36). Priscol is not only antiadrenergic but also histaminergic, cholinergic and sympathomimetic (5). Under propitious circumstances, ergotamine, yohimbine and the benzodioxane compounds of Fourneau can even be transformed from sympathetic antagonists to sympathetic synergists. Such facts as these are cited to indicate

TABLE 1 CLASSIFICATION OF DRUGS BLOCKING AUTONOMIC EFFECTOR SYSTEMS INNERVATED BY

| CHOLINERGIC NERVES  |   | ADRENERGIC NERVES   |
|---|---|---|
| Preganglionic   | Postganglionic  |   |
| High doses of<br>-choline esters<br>-anticholinesterases    | Atropine<br>Scopolamine<br>Other belladonna alkaloids | Ergot alkaloids<br>Yohimbine<br><br>933F, 883F and other Fourneau compounds |
| Nicotine (2nd phase)<br>d-Tubocurarine<br>Beta erythroidine | Synthetic 'belladonna' alkaloids<br><br>Dibutoline    | Dibenamine and congeners<br><br>Priscoland congeners                        |
| Tetraethylammonium  |   |   |
| Ganglia<br>Skeletal muscle<br>Adrenal medulla               | Smooth muscle<br>Cardiac muscle<br>Exocrine glands    |   |

After Goodman and Gilman, 1948

the complexities of the field. Indeed, a desultory examination of the massive literature on the mechanism and site of action of adrenergic blocking drugs leaves one with a sense of confusion which is aggravated, rather than dispelled, by a more detailed study (see 7, 8, 24, 33, 36, 52).

For the reasons mentioned, the older drugs available for adrenergic blockade are so far from ideal that their clinical use has been quite limited and the results obtained are generally unimpressive, even in the laboratory, as experimental tools, they have not been wholly satisfactory.

# HISTORY AND CHEMISTRY OF THE DIBENAMINE SERIES

Considerable interest therefore centers in a new series of adrenergic blocking agents which seem to possess the previously mentioned attributes to a high degree. Inasmuch as Dibenamine was the first to be discovered and has been subjected to more detailed study, it may be considered as the prototype of the series and attention will be focussed mainly on it.

N,N-dibenzyl- $\beta$ -chloroethylamine (Dibenamine) hydrochloride was originally described by O. Eisleb in 1930. It was first prepared in this country by Dr. William Gump, Senior Research Chemist of Givaudan-Delawanna, Inc., who also synthesized a large number of its chemical

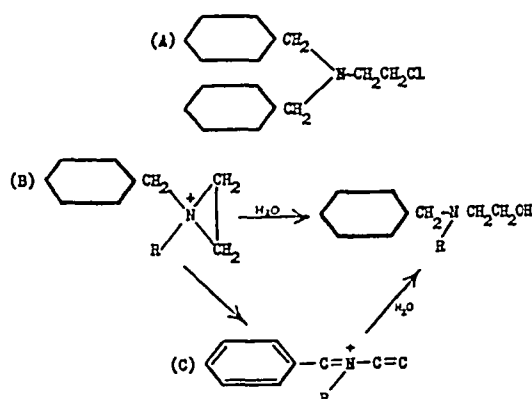


Fig. 1. 1. STRUCTURAL FORMULA of Dibenamine, N,N-dibenzyl- $\beta$ -chloroethylamine. B. Cyclization and hydrolysis of Dibenamine. C. Schematic representation of chemical structure in Dibenamine series thought to be essential for the adrenergic blocking action (see text).

congeners and related compounds. Its pharmacology was first reported by Nickerson and Goodman (39, 40, 44, 46), and subsequently studied in greater detail by Nickerson and associates (35, 36, 42, 43). When sufficient evidence had accumulated to indicate the feasibility of its use in man, Dibenamine was administered to patients by Drs. Hecht and Anderson in the Department of Medicine at the University of Utah (22). Subsequently Dibenamine was released to other laboratory and clinical investigators, and interest in the drug has steadily increased.

As the structural formula depicted in figure 1 indicates, Dibenamine is N,N-dibenzyl- $\beta$ -chloroethylamine, its name is derived by the customary process of elision. Most of the experimental work

has been done with the hydrochloride. This is a white crystalline salt, poorly soluble in aqueous media near neutrality, but soluble in aqueous acid solution, in 95 per cent ethanol and in propylene glycol. In animals, Dibenamine exerts its systemic effects by all routes of administration, but in man, for reasons to be mentioned, it must be given either intravenously or orally in enteric coated tablets.

The structural resemblance of Dibenamine to the nitrogen mustard war gases is at once apparent. It exhibits many of the chemical reactions characteristic of the nitrogen mustards, although it lacks the requisite second  $\beta$  chloroethyl grouping. The clues afforded by this fact to elucidate the specific chemical union of Dibenamine with adrenergic receptor substance are being investigated.

**Cyclization.** In aqueous solution, Dibenamine probably undergoes intramolecular rearrangement to form a cyclic ethylenimmonium cation, as shown in figure 1-B. The high chemical reactivity of ethylenimmonium cations is well known, and Dibenamine presumably exerts its pharmacodynamic effects by virtue of this intermediate product. In pure aqueous solution at physiological hydron concentrations, Dibenamine first cyclizes and then reacts with water to yield the alcohol, N,N-dibenzyl ethanolamine, which is devoid of adrenergic blocking activity. The rate of cyclization of the compound *in vitro* is relatively slow, and this may explain why the maximal effect in animals is not apparent until at least 30 minutes after injection of the drug.

**Reaction with thiosulfate.** Another chemical observation of pharmacological interest is that Dibenamine, like the nitrogen mustards, rapidly combines with thiosulfate during the stage of cyclization to form the corresponding ethyl thiosulfate. The kinetics and high specificity of the thiosulfate reaction with ethylenimmonium cation are well known and provide the basis for some interesting observations on the fate and duration of sojourn of Dibenamine in the body. For example, if a high concentration of thiosulfate is maintained in the extracellular fluid during the period of Dibenamine hydrolysis, evidence of adrenergic blockade is never exhibited. If the thiosulfate concentration is permitted to fall before the anticipated completion of hydrolysis, adrenergic blockade subsequently develops. In this manner, it has been possible to determine that the duration of sojourn of an effective dose of

Dibenamine in the tissues is of the order of 12 to 18 hours. The suggestion is advanced that the persistence of Dibenamine in the body is due to the fact that the drug is lipid-soluble, it may thus readily gain access to fat depots and be slowly released to the extracellular fluid.

**Reaction with receptor substance.** The above observations do not explain the prolonged duration of the adrenergic blockade, once it is established, which far outlasts the presence of the drug. Indeed, from three to five days may be required for full recovery from a single injection of the drug. Adrenergic blockade is probably due to the establishment of a firm chemical linkage between Dibenamine and the adrenergic receptor substance. The probability is great that the receptor substance must be reactivated, repaired or even regenerated before the excitatory response of the effector cell returns. An analogous phenomenon is that of the long duration of action of di-isopropyl fluorophosphate, until tissue cholinesterases are regenerated, its cholinergic effects are not fully dissipated.

**Structure-activity relations.** The relationship between chemical constitution and pharmacodynamic properties in the Dibenamine series has been explored by Nickerson and Gump (41). Their analysis of 139 Dibenamine congeners, 64 of which produce adrenergic blockade, indicates that the formation of an intermediate conjugated double bond system, as depicted in figure 1-C, may be the molecular basis of Dibenamine action. Substitutions in either the aromatic or alkyl halide portions of the molecule which interfere with this conjugation lead to a reduction or disappearance of activity, although similar substitutions which do not prevent conjugation are compatible with high activity. Steric factors are also involved, and substitutions on the aromatic nucleus which are out of the plane of the ring abolish activity.

**Relation to antihistaminics.** It is of both theoretical and practical interest that certain Dibenamine derivatives produce not only adrenergic blockade but are also antagonistic to histamine, as shown by Loew and Nickerson and their respective co-workers (1, 25, 26, 37). Indeed, some of the compounds examined in this laboratory are many times more potent in animals than are the antihistaminic drugs currently employed in therapeutics, they are also characterized by a remarkably long duration of antihistaminic action, probably attributable to the  $\beta$  chloroethyl radical

rather fleeting in action. For example, ergotamine causes not only adrenergic blockade but also stimulates the smooth muscle of blood vessels, the uterus and other organs, in addition, it has complex actions on the central nervous system (13, 36). Priscol is not only antiadrenergic but also histaminergic, cholinergic and sympathomimetic (5). Under propitious circumstances, ergotamine, yohimbine and the benzodioxane compounds of Fourneau can even be transformed from sympathetic antagonists to sympathetic synergists. Such facts as these are cited to indicate

TABLE 1 CLASSIFICATION OF DRUGS BLOCKING AUTONOMIC EFFECTOR SYSTEMS INNERVATED BY

| CHOLINERGIC NERVES  |   | ADRENERGIC NERVES   |
|---|---|---|
| Preganglionic   | Postganglionic  |   |
| High doses of<br>-choline esters<br>-anticholinesterases            | Atropine<br>Scopolamine<br>Other belladonna alkaloids | Ergot alkaloids<br>Yohimbine<br><br>933F, 883F and other Fourneau compounds |
| Nicotine (2nd phase)<br><i>d</i> -Tubocurarine<br>Beta erythroidine | Synthetic 'belladonna' alkaloids<br><br>Dibutoline    | Dibenamine and congeners<br><br>Priscoland congeners                        |
| Tetraethylammonium  |   |   |
| Ganglia<br>Skeletal muscle<br>Adrenal medulla                       | Smooth muscle<br>Cardiac muscle<br>Exocrine glands    |   |

After Goodman and Gilman, 1948

the complexities of the field. Indeed, a desultory examination of the massive literature on the mechanism and site of action of adrenergic blocking drugs leaves one with a sense of confusion which is aggravated, rather than dispelled, by a more detailed study (see 7, 8, 24, 33, 36, 52).

For the reasons mentioned, the older drugs available for adrenergic blockade are so far from ideal that their clinical use has been quite limited and the results obtained are generally unimpressive, even in the laboratory, as experimental tools, they have not been wholly satisfactory.

# HISTORY AND CHEMISTRY OF THE DIBENAMINE SERIES

Considerable interest therefore centers in a new series of adrenergic blocking agents which seem to possess the previously mentioned attributes to a high degree. Inasmuch as Dibenamine was the first to be discovered and has been subjected to more detailed study, it may be considered as the prototype of the series and attention will be focussed mainly on it.

*N,N*-dibenzyl- $\beta$ -chloroethylamine (Dibenamine) hydrochloride was originally described by O. Eisleb in 1930. It was first prepared in this country by Dr. William Gump, Senior Research Chemist of Givaudan-Delawanna, Inc., who also synthesized a large number of its chemical

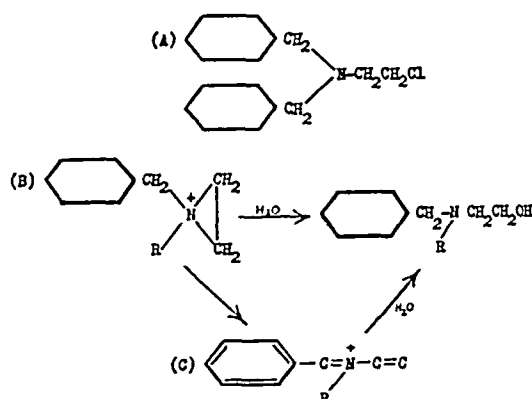


Fig. 1. 1. STRUCTURAL FORMULA of Dibenamine, *N,N*-dibenzyl- $\beta$ -chloroethylamine. B. Cyclization and hydrolysis of Dibenamine. C. Schematic representation of chemical structure in Dibenamine series thought to be essential for the adrenergic blocking action (see text).

congeners and related compounds. Its pharmacology was first reported by Nickerson and Goodman (39, 40, 44, 46), and subsequently studied in greater detail by Nickerson and associates (35, 36, 42, 43). When sufficient evidence had accumulated to indicate the feasibility of its use in man, Dibenamine was administered to patients by Drs. Hecht and Anderson in the Department of Medicine at the University of Utah (22). Subsequently Dibenamine was released to other laboratory and clinical investigators, and interest in the drug has steadily increased.

As the structural formula depicted in figure 1 indicates, Dibenamine is *N,N*-dibenzyl- $\beta$ -chloroethylamine, its name is derived by the customary process of elision. Most of the experimental work

has been done with the hydrochloride. This is a white crystalline salt, poorly soluble in aqueous media near neutrality, but soluble in aqueous acid solution, in 95 per cent ethanol and in propylene glycol. In animals, Dibenamine exerts its systemic effects by all routes of administration, but in man, for reasons to be mentioned, it must be given either intravenously or orally in enteric-coated tablets.

The structural resemblance of Dibenamine to the nitrogen mustard war gases is at once apparent. It exhibits many of the chemical reactions characteristic of the nitrogen mustards, although it lacks the requisite second  $\beta$  chloroethyl grouping. The clues afforded by this fact to elucidate the specific chemical union of Dibenamine with adrenergic receptor substance are being investigated.

**Cyclization.** In aqueous solution, Dibenamine probably undergoes intramolecular rearrangement to form a cyclic ethylenimonium cation, as shown in figure 1-B. The high chemical reactivity of ethylenimonium cations is well known, and Dibenamine presumably exerts its pharmacodynamic effects by virtue of this intermediate product. In pure aqueous solution at physiological hydron concentrations, Dibenamine first cyclizes and then reacts with water to yield the alcohol, N,N-dibenzyl ethanolamine, which is devoid of adrenergic blocking activity. The rate of cyclization of the compound *in vitro* is relatively slow and this may explain why the maximal effect in animals is not apparent until at least 30 minutes after injection of the drug.

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Speculation as to the chemical basis and pharmacological import of the concomitance of antiadrenergic and antihistaminic actions must await further experimentation

#### PHARMACODYNAMICS OF DIBENAMINE

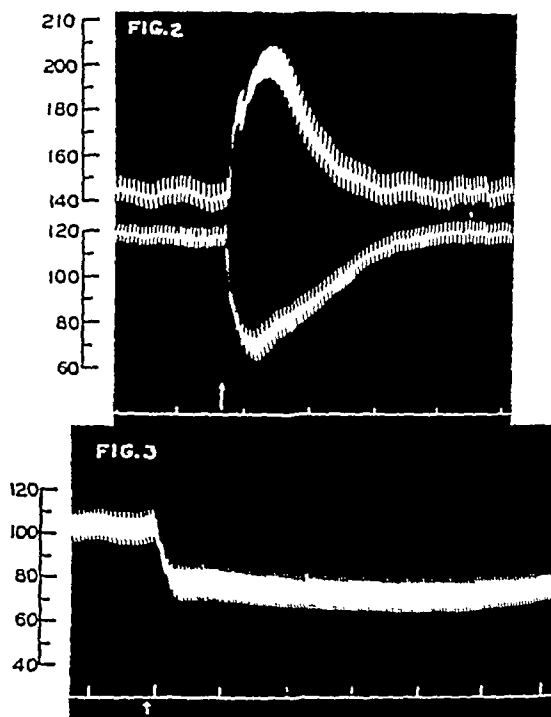
Properly administered physiological doses of Dibenamine exert only one discernible systemic action in the body, namely, blockade of excitatory adrenergic functions. Inhibitory adrenergic functions are not blocked, indeed, they may be brought into sharp relief. Dibenamine blockade is effective not only against epinephrine and circulating sympathin but also, in somewhat larger doses, against adrenergic nerve stimulation. The compound does not alter or destroy epinephrine or sympathin, as does 933F (34), and it does not prevent effector cells from responding to non-adrenergic stimulant drugs. Therefore, by a process of exclusion, the action is thought to be a direct one on adrenergic receptor substance (35, 40, 43, 53). Cholinergic mechanisms are not influenced, except indirectly by the elimination of adrenergic function in those organs which are dually innervated. Dibenamine itself exhibits only slight antihistaminic activity. Ganglionic, neuromyal and peripheral axonal transmission are apparently not affected by Dibenamine.

As so frequently is the case for autonomic drugs, the various functions of the cardiovascular system afford the best indices for observing and analyzing the actions of Dibenamine. When given slowly in full blocking doses, Dibenamine causes no significant alterations in basal heart rate, blood pressure, cardiac output or electrocardiogram of laboratory animals or resting normotensive humans. These facts suggest that rapid adjustment compensates for the loss of adrenergic tone. However, when conditions of stress are imposed, the effects of Dibenamine blockade become apparent at once.

*Reversal of epinephrine-induced blood pressure rise.* An important cardiovascular index of Dibenamine action is the response to epinephrine. Figure 2 illustrates a typical experiment on the effect of epinephrine on the carotid blood pressure of an anesthetized cat before and after Dibenamine. Dibenamine converts the pressor into a depressor response because it blocks only the excitatory vasoconstrictor effect of epinephrine and thereby unmasks the concurrent inhibitory vasodilator action. Dibenamine differs from certain other adrenergic blocking agents in that

its action is uninfluenced by anesthesia and does not vary with the type of anesthetic employed.

The completeness of the Dibenamine blockade is indicated by the fact that massive doses of epinephrine produce only pure vasodepression, as illustrated in figure 3. Even 10 milligrams per kilogram of epinephrine have been administered intravenously with results comparable to those shown in the figure. The fall in blood pressure lasts for many minutes, until the concentration of circulating epinephrine has fallen below the



Figs 2 and 3 EFFECT OF DIBENAMINE ON THE blood pressure response to intravenous epinephrine. Upper record of fig 2 before and lower record of fig 2 and fig 3 after 15 mgm/kgm Dibenamine. Epinephrine (fig 2, 2.5  $\mu$ gm/kgm, fig 3, 1000  $\mu$ gm/kgm) injected at arrows. Cat anesthetized with pentobarbital. Time in minutes, ordinate scale in mm Hg.

threshold value capable of causing vasodilatation. Depressor responses have been obtained with pressures as low as 30 mm of Hg. In the rabbit, Dibenamine blocks but does not reverse the pressor action of epinephrine. This species apparently lacks an adrenergic vasodilator system (10).

Whereas the pressor response to epinephrine varies directly with dosage over a wide range, the depressor response is definitely limited in magnitude and is fully elicited by relatively small amounts of epinephrine. Figure 4 plots the rise or

fall of blood pressure against the dose of epinephrine. An increase in dose of epinephrine beyond 5 micrograms per kilogram causes no further fall in pressure, although the duration of the fall is prolonged when the larger amount of epinephrine must be eliminated. Obviously the response of the adrenergic vasodilator system is limited, but its activation by low concentrations of epinephrine provides a rational explanation for the secondary, depressor phase of epinephrine action.

Neither Dibenamine, nor the ergot alkaloids nor the 'adrenolytic' drugs of the Fourneau series prevents the chronotropic and the positive inotropic effects of epinephrine on the heart (2, 23, 40). Thus, after Dibenamine epinephrine still

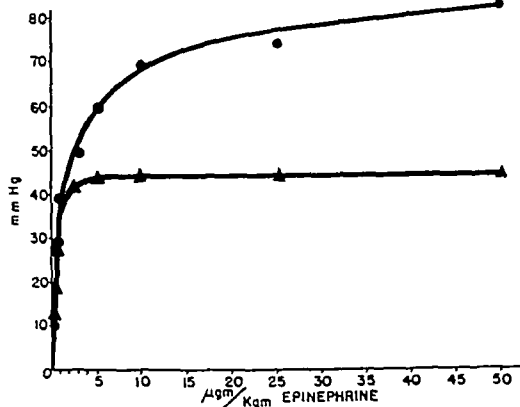


Fig 4 RELATION OF THE DOSE OF EPINEPHRINE (intravenous) to the Hypertension (upper curve) elicited before and the hypotension (lower curve) elicited after the administration of Dibenamine. Data averaged from 6 cats. Ordinate, mm Hg blood pressure, abscissa, dose of epinephrine in  $\mu\text{gm}/\text{kgm}$ .

elicits an increase in sinus rate, stroke volume and cardiac output. Apparently the action of epinephrine on the sino-aortic node and on the force of myocardial contraction is fundamentally different from that on vascular smooth muscle.

**Effect on splanchnic nerve stimulation.** Dibenamine is not only a potent 'adrenolytic' but also a potent 'sympatholytic' drug. Thus the compound blocks and reverses the excitatory responses not only to epinephrine but also to sympathetic nerve stimulation. In figure 5 is illustrated the blood pressure response of a cat to electrical stimulation of the resected splanchnic nerve. The initial sharp component of rise in pressure, seen in the topmost tracing (A), is the result of direct sympathetic stimulation of the splanchnic vascular bed. The subsequent slower component of the rise is the

result of the release of epinephrine and some sympathin into the systemic circulation, and is largely abolished by removal of the adrenal glands. The middle tracing (B) is that of the blood pressure response to splanchnic nerve stimulation in the same animal after Dibenamine. Each of the two components of the original pressor response is reversed. The initial rapid fall is rather small, which indicates that splanchnic vasodilatation is limited in the cat, as emphasized by Cannon and Rosenblueth (11). The second and slower component of the reversal represents the vasodilator response to endogenously released epinephrine. The lowermost tracing (C) illustrates the Dibenamine blockade of the response to splanchnic stimulation in the same animal after removal of the adrenal glands.

**Effect on direct and reflex sympatho-adrenal discharge.** Splanchnic nerve stimulation causes only regional sympathetic nerve discharge. Generalized sympatho-adrenal discharge can be elicited in various ways, one of which is shown in figure 6. If an atropinized cat is injected with a large dose of a choline ester having nicotinic properties, such as carbachol, a biphasic blood pressure rise is elicited. Atropine prevents the vasodilator and cardiodecelerator responses to carbachol, so that the stimulatory effects of the ester on sympathetic ganglia and on the adrenal medulla stand out in bold relief. The upper curve in the left-hand portion (A) of figure 6 shows the two characteristic components of the sympatho-adrenal discharge caused by carbachol. The lower tracing represents the reversal of the response in the same animal, after Dibenamine. The two curves are almost mirror images of each other. The right-hand portion (B) of figure 6 depicts the same experiment in an adrenalectomized animal before and after Dibenamine. Here the pressor response is caused only by sympathetic ganglionic discharge and it is characteristically brief in duration. Its reversal by Dibenamine is evident. The experiment clearly indicates that Dibenamine can reverse the pressor effect of generalized direct discharge of the sympathetic nervous system, both in the presence and in the absence of the adrenal glands.

Another way to evoke widespread sympatho-adrenal discharge is by reflex means, for example, by anoxia, as shown in figure 7. Gradually developing anoxia was produced by the use of a small rebreathing bag and a soda-lime canister. The upper tracing illustrates the vasopressor effect in an anesthetized cat of two short periods

of anoxia. The lower tracing depicts three pure vasodepressor responses to anoxia in the same animal, after the injection of Dibenamine. It is

As shown by Nickerson and associates (40, 42, 45, 46), Dibenamine also manifests marked ability to prevent ventricular rhythms evoked by

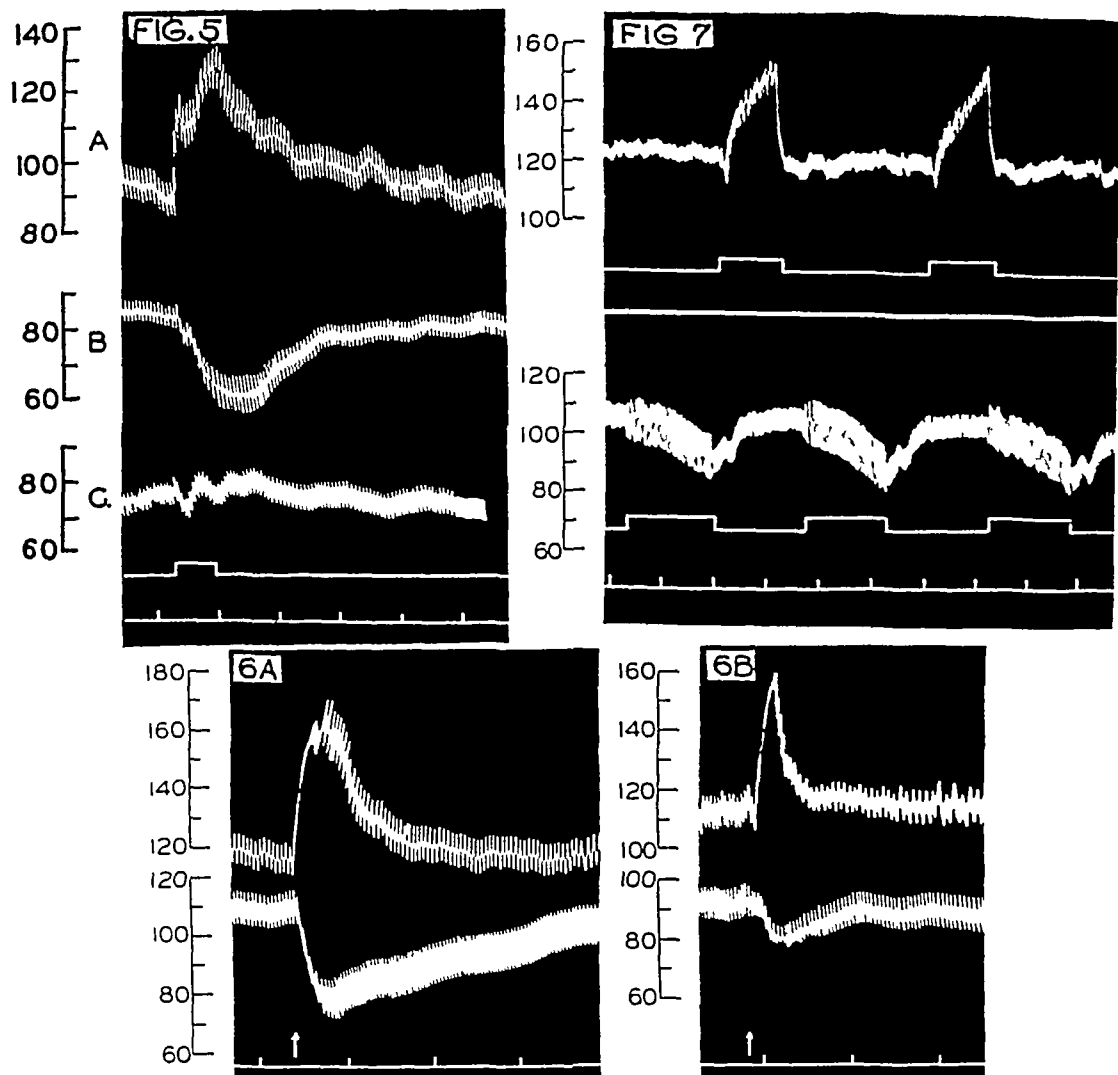


Fig 5 BLOOD PRESSURE RESPONSE TO SPLANCHNIC NERVE STIMULATION. Cat under urethane anesthesia. The splanchnic nerves were resected bilaterally just above the diaphragm. Electrodes on distal stumps of right splanchnic nerves. Period of stimulation indicated by upward deflection signal line. Variable frequency, square-wave electronic stimulator, 20 pulses/sec and pulse width 0.5 msec, current employed was that necessary for maximal response in control animal. Time in minutes, ordinate scale, mm Hg. A before and B after Dibenamine, C after Dibenamine and removal of adrenal glands.

Fig 6 BLOOD PRESSURE RESPONSE TO THE INTRAVENOUS ADMINISTRATION OF CARBACHOL IN AN ATROPINIZED CAT UNDER PENTOBARBITAL ANESTHESIA. A Upper record before and lower record after intravenous administration of 15 mgm/kgm Dibenamine. Carbachol injected at the arrow, time in minutes, ordinate scale, mm Hg. B Same procedures as in A, after bilateral adrenalectomy.

Fig 7 BLOOD PRESSURE RESPONSE TO ANOXIA BEFORE (upper record) and after (lower record) Dibenamine. Cat under pentobarbital anesthesia. Periods of anoxia are indicated by upward deflections of signal line. Time in minutes, ordinate scale, mm Hg.

thus obvious that Dibenamine also blocks the response to reflexly elicited sympathetic discharge.

*Prevention of cyclopropane-epinephrine arrhythmias*

in animals under cyclopropane anesthesia. The protection afforded by Dibenamine is almost absolute and is far greater than that provided by isonipicaine, atropine, bilateral vi-



gotomy, procaine, quindine, Priscol or orgotamine. In the Department of Anesthesia at the University of Utah, these observations have been extended to man. Dibenamine can reduce or abolish ominous types of ventricular rhythms occurring in surgical patients under cyclopropane anesthesia.

Figure 8 illustrates typical experiments in dogs fully equilibrated to 30 per cent cyclopropane, 70 per cent oxygen and injected intravenously with 10 micrograms per kilogram of epinephrine, according to the standardized technique developed by Meek, Orth and their associates at the University of Wisconsin (31, 32, 48). The ECG was recorded

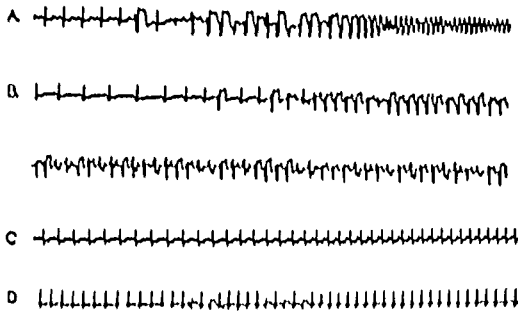


Fig 8 PROTECTIVE ACTION OF DIBENAMINE against epinephrine induced cardiac arrhythmias in cyclopropanized dogs. Dogs equilibrated to 30% cyclopropane 70% oxygen and injected intravenously with epinephrine 10  $\mu$ gm/kgm, over a period of 50 seconds. ECG recorded by thermo writing oscillograph. A Control ventricular extrasystoles, multiple focus ventricular tachycardia, and death from ventricular fibrillation. B Control ventricular extrasystoles, multiple focus ventricular tachycardia and recovery. C and D Epinephrine injected 30 minutes after Dibenamine, 20 mgm/kgm. Sinus tachycardia only (C) and sinus tachycardia with occasional ventricular extrasystole (D).

by a thermo writing oscillograph. Tracing A is that of a control dog which developed ventricular extrasystoles and tachycardia, and fatal ventricular fibrillation after epinephrine. Tracing B is that of a control dog which exhibited ventricular extrasystoles and tachycardia, but survived. Tracings C and D are typical of those obtained in dogs protected by Dibenamine. Sinus tachycardia uniformly occurs. Although occasional ventricular extrasystoles are noted in some experiments, serious ventricular rhythms do not develop. From 100 to 500 times the standard amount of epinephrine can be given without the occurrence of cardiac effects significantly different than those

noted from the 10 microgram dose. Inasmuch as sinus tachycardia and increased cardiac output are not prevented by Dibenamine, it is obvious that the mechanism of ventricular fibrillation induced by epinephrine is not intimately related to the chronotropic and motropic actions of the latter drug on the heart.

Of the various drugs and procedures which have been employed to protect the heart against epinephrine in cyclopropanized dogs, only Priscol approaches Dibenamine in efficiency. When the standard test dose of 10 micrograms per kilogram of epinephrine is injected in dogs, both Priscol and Dibenamine prevent ventricular tachycardia and fibrillation, and greatly reduce the period of total cardiac irregularity (table 2). However, when the standard dose of epinephrine is increased only tenfold, that is, to 100 micrograms per kilogram, the efficacy of Priscol drops sharply whereas that of Dibenamine remains unaffected. Larger doses of both Dibenamine and Priscol are required to protect against cyclopropane epinephrine cardiac arrhythmias than to reverse the pressor action of the same dose of epinephrine.

*Effect on experimental hypertension and experimental shock.* A detailed presentation of the effects of Dibenamine therapy on experimental renal hypertension in animals is purposely omitted from this discussion. As is also true for the use of other adrenergic blocking drugs, the reported results are inconclusive (38, 40, 55). Dibenamine has also been utilized in animal experiments by Wiggers and co-workers (51, 54) in order to ascertain the rôle played by sympathetic vasoconstriction in crucial organs in the development of irreversible hemorrhagic shock. By preventing compensatory reflex vasoconstriction, Dibenamine permitted a significantly higher incidence of survivals than occurred in the control series. These observations are in agreement with the results of similar experiments by Nickerson and Price (30).

*Other manifestations of adrenergic blockade.* Other features of adrenergic blockade by Dibenamine are as follows. The intact animal exhibits ptosis of the eyelid, marked extension of the nictitating membrane and miosis. Indeed, in man, pupillary constriction is the earliest and most sensitive indicator of Dibenamine's action. The mydriasis, widening of the palpebral fissure and retraction of the nictitating membrane, in response either to epinephrine or to cervical sympathetic nerve stimulation, are largely blocked by Dibenamine. The effect of Dibenamine on the ciliary body and accommodation is not yet

known, the drug may provide a useful means for determining whether sympathetic nerves are at all concerned with accommodation. The pilot-motor response to sympathetic nerve stimulation is abolished. The erythremia and leukocytosis occurring during excitement and resulting from contraction of the spleen are prevented. Although denervated adrenergic effector cells are exquisitely sensitive to epinephrine, they fail to respond to 100 or more times the effective dose, if Dibenamine is given beforehand. Dibenamine markedly elevates the lethal dose of epinephrine (26, 40, 49). This protective action may permit the parenteral administration of sufficiently large amounts of the hormone to enable its fate and excretion to be studied with facility, and thus to

glands follow the peripheral neural pathways of the sympathetic system, sweating in man is usually considered to be a cholinergic and not an adrenergic function. Nevertheless, certain sympathomimetic amines are known to induce sweating, the failure of epinephrine to do so is usually explained by the coexisting local vasoconstriction. Quantitative measurements of spontaneous palmar sweating in man have been made by Humovic (20) who observed that Dibenamine caused marked inhibition. In addition, sweating induced by neo-synephrine was completely prevented by Dibenamine. This investigator interprets these facts to indicate that adrenergic sweating is a physiological phenomenon, at least in certain cutaneous areas. In any event, Diben-

TABLE 2 EFFICACY OF DIBENAMINE AND PRISCOL IN PREVENTING EPINEPHRINE-INDUCED CARDIAC ARRHYTHMIAS IN CYCLOPROPANIZED DOGS

| TREATMENT   | DOSE<br>MGM<br>KGM | NO. ANIMALS | V F | TOTAL CARDIAC<br>IRREG<br>in sec | C <sub>O</sub> CHANGE | V T<br>in sec | C <sub>O</sub> CHANGE |
|---|--------------------|-------------|-----|----------------------------------|-----------------------|---------------|-----------------------|
| <i>Standard test dose epinephrine, 10 µgm/kgm</i> |                    |             |     |                                  |                       |               |                       |
| Control   | —                  | 25          | 32% | 145 ± 7                          | —                     | 91 ± 9        | —                     |
| Dibenamine  | 20                 | 17          | 0%  | 3 ± 1.7                          | -98                   | 0             | -100                  |
| Priscol   | 20                 | 6           | 0%  | 3 ± 2.0                          | -98                   | 0             | -100                  |
| <i>Test dose epinephrine, 100 µgm/kgm</i>         |                    |             |     |                                  |                       |               |                       |
| Dibenamine  | 20                 | 17          | 0%  | 3 ± 1.8                          | —                     | 0             | —                     |
| Priscol   | 20                 | 6           | 17% | 65 ± 15                          | —                     | 25 ± 8.4      | —                     |

Dogs were equilibrated to 30% cyclopropane 70% oxygen and the ECG recorded continuously with a thermo-writing oscillograph. The standard test dose of epinephrine, 10 µgm per kilogram, was then injected intravenously at a uniform rate during a period of 50 seconds. Ten times this standard dose was also employed. V F, ventricular fibrillation; V T, ventricular tachycardia. Mean duration of arrhythmia is given in seconds ± its standard error.

permit extension of the accumulating evidence (6, 14, 50) that the pathway of inactivation of epinephrine is conjugation with sulfate and subsequent renal excretion, rather than enzymatic oxidative destruction.

Most of the properties thus far described for Dibenamine pertain to smooth and cardiac muscle. But both sympathin and epinephrine act on other than muscular systems in the body. Therefore the influence of Dibenamine on these systems requires comment. Unfortunately, the evidence for blockade of exocrine glandular secretions in animals is as yet incomplete. In man, however, Dibenamine has been observed to inhibit sweating. This is a most important finding. Despite the fact that the fibers innervating sweat

glands follow the peripheral neural pathways of the sympathetic system, sweating in man is usually considered to be a cholinergic and not an adrenergic function.

*Inability to block inhibitory adrenergic systems.* A feature common to all adrenergic blocking drugs is that, in general, they are unable to reverse or prevent the *inhibitory* effects of adrenergic nerve impulses or of epinephrine. Although exceptions to this generalization have been reported, the observations are few in number and leave much to be desired with regard to careful control and analysis. The evidence that Dibenamine does not block or reverse inhibitory sympathetic functions is as follows: adrenergic vasodilatation is particularly prominent after Dibenamine, the pure vasodepression caused by Isuprel

(*N*-isopropyl in log of nor epinephrine which lacks the excitatory component of vascular action) is not prevented, and smooth muscles which are relaxed by epinephrine or sympathetic stimulation are uninfluenced. Figure 9 illustrates the inability of Dibenamine to block the relaxation induced by epinephrine in the non-pregnant cat uterus *in situ*. The two upper tracings show the vasopressor and vaso-depressor responses to epinephrine, before and after adrenergic blockade. The two lower tracings show that uterine relaxation occurs equally well, before and after Dibenamine. In contrast to the cat uterus, the rabbit uterus is contracted by epinephrine, *in vivo* and *in vitro*. This contraction is both prevented and reversed by Dibenamine. The intestine relaxes in response to epinephrine despite Dibenamine, and

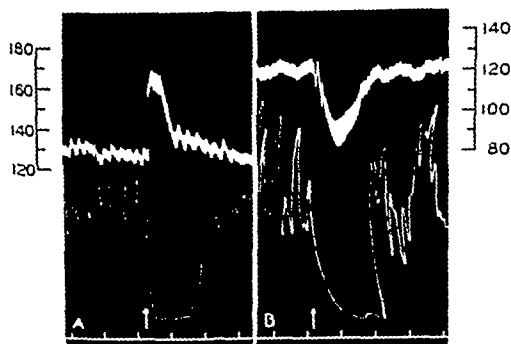


Fig 9 RESPONSE OF THE BLOOD PRESSURE and the uterus (*in situ*) in a non pregnant cat (1) before and (B) after Dibenamine. Downward deflections in lower tracings indicate uterine relaxation. The arrows indicate intravenous injection of 25  $\mu\text{gm/kgm}$  epinephrine. Time in minutes, ordinate scale, mm Hg.

presumably the bronchial musculature does likewise. It is thus established that only adrenergic excitatory systems are blocked by this class of drugs. If and when agents are discovered which produce specific blockade of adrenergic inhibitory systems, some insight may be gained into the basic differences between excitatory and inhibitory sympathetic receptor mechanisms.

**Metabolic, respiratory and other systems.** It is well known that the metabolic effects of epinephrine are not significantly influenced by adrenolytic drugs. However, ergotamine and dihydro-ergotamine are reported to block the epinephrine-induced rise in blood sugar (52). Dibenamine, in contrast, does not prevent this rise in blood sugar. Nor are the respiratory effects of epinephrine altered. For example, the characteristic but

transient 'epinephrine apnea', mediated through the carotid chemoreceptor system, is not blocked by Dibenamine in doses which readily reverse the blood pressure response (fig 10). Also hyperventilation, evoked by large doses of epinephrine and probably central in origin, is not prevented by Dibenamine. If sympathin and epinephrine exert a physiological effect on ganglionic and neuromuscular transmission, as has been claimed (9, 29, 30), it seems most unlikely that Dibenamine blocks their action at these loci; however, the problem deserves closer examination.

**Effect on other sympathomimetic amines.** It is of both academic interest and practical importance

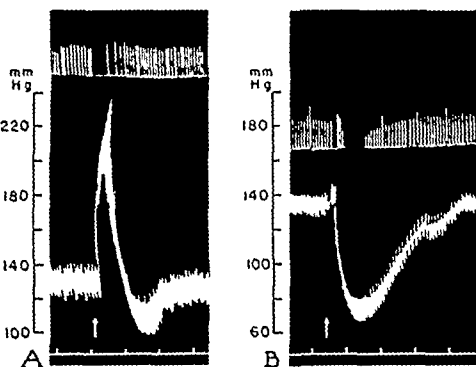


Fig 10 EFFECT OF EPINEPHRINE on respiration (upper tracing) and blood pressure (lower tracing), before (A) and after (B) Dibenamine. Dog, anesthetized with pentobarbital sodium. A: At the arrow, epinephrine was injected intravenously, 5  $\mu\text{gm/kgm}$ , and elicited the typical blood pressure response and transient apnea, the latter resulting from carotid chemoreceptor stimulation. Between A and B, Dibenamine was administered, 15  $\text{mgm/kgm}$ , and 45 minutes were permitted to elapse. B: The same dose of epinephrine was injected at the arrow. The pressor response was reversed but the transient respiratory arrest was unaffected.

to know whether other sympathomimetic amines are affected by Dibenamine as is epinephrine. The theory of Gaddum and Kwikowski (17) and others proposes that ephedrine and perhaps other pressor amines exert their sympathomimetic actions in the body by preventing the oxidative destruction of epinephrine and sympathin. If this were true, one would expect their excitatory effects to be blocked by adrenergic blocking drugs.

Dibenamine reduces the pressor responses to all 25 sympathomimetic amines tested to date, but the responses are not reversed in all cases. Amines which have the 3,4-catechol nucleus and an aliphatic substitution on either the nitrogen or

the *beta* carbon are readily blocked and reversed. Compounds with a single hydroxyl group on the aromatic nucleus are reversed with difficulty, but some vasodepressor action can be demonstrated in most cases. Finally, straight chain sympathomimetic amines, those with an unsubstituted aromatic ring, and those without an aliphatic substitution on either the nitrogen or the *beta* carbon can be reversed only under special conditions.

A fact of practical importance is that the pressor effect of certain amines, such as amphetamine and Tuamine, is only partially blocked by the usual doses of Dibenamine. By the use of such amines, it should be possible to elevate when necessary the blood pressure in patients given Dibenamine. The pressor activity of angiotonin is essentially unaffected by Dibenamine (19, 38, 56), a fact which confirms the specificity of the Dibenamine blocking action.

Since Cannon and Rosenblueth (11, 12) advanced the hypothesis of two sympathins, the possibility that sympathin E is in reality nor-epinephrine has received ever-recurrent attention, in more recent times particularly by Gaddum and Goodwin (16) and by Euler (15). If the theory is correct, adrenergic drugs should block but not reverse the pressor action of nor-epinephrine, and indeed this is the case. However, such evidence is merely contributory and not conclusive, and there are reasons not to accept the theory.

#### PHARMACOLOGICAL ACTIONS IN MAN

The pharmacological actions of Dibenamine in man (21, 22, 23) are essentially the same as those observed in laboratory animals, and may be summarized briefly. The effects of a single injection persist for 36 to 96 hours. Resting blood pressure, heart rate and ECG in normotensive individuals are not significantly altered. As has been shown by Hamovici and Mednits (21), Dibenamine lowers the blood pressure toward normal in patients with early or moderately advanced essential hypertension, but not in patients in the malignant phase of the disease. As illustrated in figure 11, a single infusion of Dibenamine in a subject with moderately advanced essential hypertension causes a significant reduction in blood pressure, lasting many hours. The reduction can be maintained if Dibenamine is administered at appropriate intervals, but the therapeutic significance of this manometric success cannot be stated at present. Significant alter-

ation in basal heart rate is not observed. Orthostatic hypotension occurs in both normotensive and hypertensive individuals and is elicitable for 12 to 24 hours after the drug. Postural tachycardia persists even longer. In the figure, the vertical broken line at the fifth hour depicts the fall in blood pressure which occurred when the patient assumed the upright position from recumbency.

Dibenamine increases peripheral blood flow and elevates skin temperature, particularly in patients with neurogenic vascular spasm of the extremities. Figure 12 illustrates the marked rise in cutaneous temperature of the toes following a single infusion of Dibenamine in a 35 year old patient with severe essential hypertension (22). In such advanced cases the systemic blood pressure usually does not fall.

The pressor response to intravenous epinephrine in man following Dibenamine is greatly reduced or reversed as it is in animals. However, the sinus tachycardia, enhanced cardiac output, increased rate of blood flow and T-wave changes normally produced by epinephrine are not blocked (22). The customary hemodynamic responses to the cold pressor, breath-holding and Flick tests are either markedly diminished, or abolished or reversed. Neo-synephrine injected intravenously in man causes a marked compensatory bradycardia secondary to the elevation of blood pressure, by reducing or preventing the rise in blood pressure, Dibenamine abolishes the neo-synephrine bradycardia.

Arrhythmia is elicitable in man by either cyclopropine or epinephrine are decreased or prevented. Dibenamine in appropriate doses lowers the incidence and shortens the duration of ominous ventricular rhythms which occur in patients in the deeper planes of cyclopropane anesthesia. After Dibenamine, A-V nodal rhythm and occasional ventricular extrasystoles ordinarily comprise the only abnormalities observed. As was noted in animals, larger doses of Dibenamine are required to prevent cardiac arrhythmias in man than to block or reverse most excitatory responses to adrenergic stimulation.

Miosis appears early and persists for the duration of action of Dibenamine. Nasal mucosal congestion is also common, it is probably caused by local vasodilatation from adrenergic blockade. Spontaneous sweat secretion is inhibited in some areas of the body. The functions of the respiratory, gastrointestinal, genitourinary and somatic neuromuscular systems do not appear to be sig-

nificantly modified. Body temperature is unaltered and the blood picture is unchanged. As yet, careful studies of the action of Dibenamine on renal hemodynamics and renal function have not been reported.

### TOXICITY

The toxicity of Dibenamine is manifested in two different ways, by local tissue damage and by stimulation of the central nervous system (21, 22, 40). Although less irritating than the nitrogen mustards, Dibenamine can cause local tissue de-

amine appears adequate and cumulative toxicity from repeated clinical doses has not been observed.

When Dibenamine is injected too rapidly by the intravenous route, evidence of central excitation occurs in both animals and man. In some patients, confusion, emotional lability, restlessness, nausea and vomiting have been observed. These side effects are transient. A slow rate of infusion or preliminary barbiturate sedation usually decreases or prevents these central manifestations. The central stimulant action of Diben-

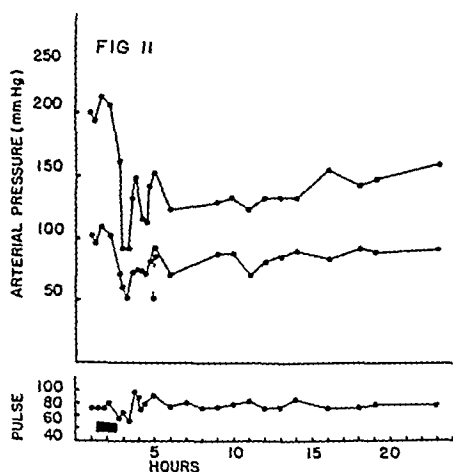
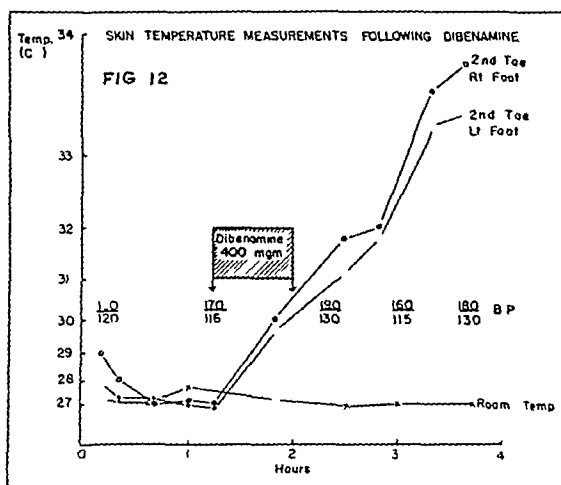


Fig 11 EFFECT OF DIBENAMINE on the blood pressure and pulse rate of a hypertensive subject. The patient had moderately advanced but benign essential hypertension. Dibenamine 5 mgm/kgm was infused intravenously during the interval shown by the black rectangle. Observe the extent and long duration of the fall in systolic and diastolic blood pressures, and the absence of significant effect on cardiac rate. The vertical broken line at the 5th hour indicates the extent of fall of the blood pressure upon the patient's assuming the upright position from recumbency (After Haimovici and Mednits, 1948).

Fig 12 EFFECT OF DIBENAMINE on cutaneous temperature in man. Temperature was measured by means of thermocouples on the right and left second toes, before, during and after the slow intravenous infusion of 5 mgm/kgm Dibenamine in a 35 year old male with severe essential hypertension. The patient was resting without covers in a warm room. Note the rise in skin temperature and the absence of significant changes in the blood pressure (After Hecht and Anderson, 1947).



struction. Parenteral administration in man is permissible only by the intravenous route. Paravenous injection must be avoided and certain precautions observed to prevent local phlebotrombosis. Gastrointestinal irritation from oral administration of the drug occurs in some but not in all patients and may interfere with therapy by this route; indeed, the response to oral medication is rather inconstant and unpredictable. Dibenamine exhibits none of the hemotoxic properties so characteristic of the nitrogen mustards, probably because it possesses only a single  $\beta$ -chloroethyl grouping. The therapeutic ratio of Diben-

amine is quite unrelated in time and in mechanism to the adrenergic blocking action. Indeed, almost identical central effects are caused by the hydrolysis product, *N,N*-dibenzyl-ethanolamine (40); this alcohol exhibits no adrenergic blocking activity.

A peculiar psychic feature of the central effect of Dibenamine deserves comment. In some patients, there occurs a type of transient repetitive temporal hallucination or reduplicative paramnesia in which an event seems to have been already experienced at the very moment when it is being experienced. With regard to the visual

component, this is the well-known phenomenon of *déjà vu*. Psychiatrists may find Dibenamine a valuable drug for investigating the nature and significance of this fascinating psychic reaction.

#### THERAPEUTIC POTENTIALITIES

The therapeutic potentialities of Dibenamine require brief mention. If a new drug is not to remain merely an interesting curiosity or a useful laboratory tool, it must have clinical potentialities commensurate with its pharmacodynamic properties. On the basis of its pharmacological actions, Dibenamine and its congeners should be of value in conditions in which partial or complete blockade of excitatory adrenergic functions is indicated for diagnostic purposes or for symptomatic or specific therapy. These conditions, to mention only the more obvious, would include peripheral vascular diseases, such as thrombophlebitis, acute arterial occlusion, frostbite, causalgia, Raynaud's disease, organic vascular insufficiency with components of functional spasm, etc., and certain phases of hypertensive disease, particularly refractory headache and hypertensive crises such as acute encephalopathy and retinopathy. In addition, Dibenamine may prove useful in the diagnosis of pheochromocytoma and in ameliorating symptoms of the associated hypertensive crises, and it may afford protection against serious ventricular rhythms occurring during general anesthesia, especially with cyclopropane. The drug may also prove serviceable in the selection of those patients with hypertension or other syndromes, most likely to experience a salutary response to generalized or regional sympathectomy, and as a test for the completeness of sympathetic neurectomy. Indeed, clinical results to date, although still limited, suggest that Dibenamine may be of value for a number of the purposes mentioned.

It is obvious that, in general, the field of usefulness of the drug overlaps that of tetraethylammonium bromide, Priscol, paravertebral local anesthetic block and surgical resection. Conceiv-

able advantages of Dibenamine over other methods of interrupting sympathetic efferent pathways include its specificity of action, its prolonged effect, its blockade of all vascular beds simultaneously, the avoidance of sensitization to epinephrine and sympathin, the reversible nature of its action, and the lack of effect on sympathetic vasodilator and inhibitory functions.

For the present, the intravenous administration of Dibenamine is a procedure restricted to hospitalized patients. The drug is being investigated in a number of university hospitals and clinics, and one may anticipate that the results of these studies will determine the value and limitations of Dibenamine and delineate its status in comparison with other therapeutic agents and procedures.

#### SUMMARY

In summary, it may be stated that a new series of potent, specific and long-acting adrenergic blocking agents has been developed which has academic and heuristic value for investigating the physiology of the sympathetic nervous system, and which provides a fresh approach for such investigations. In addition, the most carefully studied member of the group has been employed clinically with encouraging preliminary results in a variety of diseases and syndromes in which therapeutic peripheral blockade of the excitatory adrenergic system is indicated.

The discovery of the Dibenamine series has raised many questions which are as yet unanswered. Pharmacologists are the first to recognize that the use of a new drug as a physiological tool frequently raises more problems than it solves. Whatever else the discovery of this group of drugs may accomplish, it has renewed the interest in the field of adrenergic blockade. Experimental and clinical applications of Dibenamine and its congeners may reasonably be expected to yield new and valuable information on the normal and pathological physiology of the sympathetic nervous system.

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# NUTRITIONAL SIGNIFICANCE OF THE INTESTINAL FLORA

C. A. ELVEHJEM

*Department of Biochemistry, University of Wisconsin*

Ten years ago considerable skepticism was encountered when attempts were made to explain variations in the nutritional requirements of different species of animals on the basis of intestinal synthesis of vitamins. Today there is a tendency to use this explanation for many of the results that are not readily understood in nutrition studies. Since new approaches to a problem are often over-emphasized after the original resistance has been overcome, I should like to discuss in an impartial manner the nutritional significance of the intestinal microflora in light of our present knowledge.

The term 'nutritional significance' is used for a specific purpose because the organisms may be responsible for both synthesis and destruction of nutrients, for production and inactivation of toxic agents, as well as for alterations in the digestive mechanisms.

The term 'intestinal flora' is not so satisfactory since it suggests that all the organisms present can be identified and measured quantitatively. Unfortunately, such a tabulation is impossible and preliminary studies indicate that much more work is needed before the entire population can be accounted for. Furthermore, little attention has been given to the variations encountered in different parts of the intestinal tract.

We must admit from the beginning that one of the important approaches to the problem involves the use of germ-free animals and animals in which specific flora are established. Studies in this field have been reviewed by Glumstedt (1) and by Reyniers (2). It appears that this work will be expanded in the near future, but in the meantime we will have to rely largely upon the indirect approach. Actually, this phase of the problem may have greater practical application because all humans and most animals must live in the presence of a multiple flora. If we are to make use of the bacterial activity we are dependent upon those organisms already established in the tract, although eventually it may be possible to modify the flora to the advantage of the host.

Time does not permit an historical resume, but it is interesting to note a few of the significant early contributions. Probably the first to observe

the importance of intestinal bacteria in nutrition from the modern view point were Osborne and Mendel (3) in 1911. Their paper has become a classic, not because of the specific subject studied, the comparative nutritive value of various proteins, but because it introduced the use of the rat in nutritional problems and gave further evidence for the existence of accessory food substances. They found that rats which had been maintained for long periods of time on isolated food stuffs became koprophagists especially when deficiency symptoms developed. In nearly every case the occasional addition of a small amount of feces from a normally fed rat stopped the decline in weight of the deficient animals. Cooper (4) and Portier and Randoim (5) detected vitamin B in the feces of chickens and rabbits by feeding the feces to polyneuritic pigeons.

Further interest in this problem developed during 1923-25 when several workers (6, 7, 8) attempted to improve the biological assays for vitamin B. Steenbock, Sell and Nelson (6) observed that rats grown on raised screens required at least twice the amount of a supplement to prevent vitamin B deficiency as those having their feces always available. Heller *et al* (8) and Salmon (9) emphasized that it was even more important to prevent the consumption of feces when the rats were given a roughage or certain natural foods. Shortly after these studies Friedericia (10), Roscoe (11) and Kon and Watchhorn (12) described a phenomenon whereby rats which had been depleted on a B complex deficient diet spontaneously resumed growth and normal appearance without the administration of vitamin containing supplements. These spontaneous recoveries were obtained in rats fed rations containing a large amount of uncooked starch and were accompanied by the production of bulky gas occluded white feces. Friedericia called this phenomenon *refection* from the Latin *reficere*, to restore. Although some workers have failed to produce *refection*, many workers have now described the condition (reviewed by Kelly and Parsons, 13) and the original interpretation remains unchanged.

How the relected rats obtain the vitamins of



bacterial origin, whether through vicarious practice of coprophagy or by direct absorption from the tract supposedly in the region of the cecum, has not been clearly determined. In ruminants where synthesis of most of the B vitamins has been demonstrated there can be no question regarding the direct use of the vitamins, but in these animals the rumen contents pass into regular digestive channels. Much effort has gone into attempts to prevent coprophagy in rats but complete elimination has rarely been obtained. We (14) have recently developed a so called tube cage which effectively prevents the consumption of feces, but it has not been used in cases of refection.

Guerrant and Dutcher (15) reported that only the feces from dextrin-fed rats had any beneficial effect when fed to deficient animals, that the cecal contents were more active than the material from the rest of the tract and that yeast cells were probably responsible for the synthesis of the vitamins. Morgan, Cook and Davison (16) found that lactose favors production of riboflavin and pyridoxine and that corn starch favors the production of the filtrate factor (probably pantothenic acid).

Lamoreau and Schumacker (17), working with chicks, found twice as much riboflavin in the excreta as in the feed if no precautions were taken in collecting the feces. However, if the feces were placed directly in alcohol no more riboflavin occurred in the excreta than could be accounted for by the feed. Actually there was a 100 per cent increase in the riboflavin content of the feces when held at room temperature for several days. These results, together with the fact that the young chick readily develops a vitamin K deficiency while rats can rely upon intestinal synthesis of vitamin K, led to the idea that vitamin production in the tract of chicks is very limited. However, this may not be true in the case of all the essential factors.

Let us now turn to experiments conducted with more highly purified rations. The composition of a typical ration now widely used is given in table 1. Weanling rats placed on this diet will grow 25-30 grams per week over a six-week period. Actually five of the 10 B vitamins listed can be omitted without affecting the rate of growth. Of these five vitamins, four certainly must be produced through some synthetic mechanism because the total amount in the rat increases with the growth of the animal. The best evidence for intestinal synthesis is available in the case of folic acid and biotin. If folic acid is omitted from the ration and

0.5 to 2 per cent of succinylsulfathiazole or phthalylsulfathiazole is added, poor growth and granulocytopenia develop (18). Results tabulated in table 2 show that the amount of folic acid in the liver (19) and in the cecal contents (20) decreases when the drug is fed. Some of the folic acid must be absorbed directly from the cecum, since rats kept in tube cages show approximately the same amount of folic acid in the liver as those kept in ordinary cages. However, rats kept on a synthetic ration with the five B vitamins show a greater growth response to both folic acid and biotin.

TABLE 1 A TYPICAL PURIFIED RATION

|  | gm |                     | mgm   |
|--|----|---------------------|-------|
| Sucrose  | 73 | Thiamine            | 0.2   |
| Casein   | 18 | Riboflavin          | 0.3   |
| Corn oil   | 5  | Pyridoxine          | 0.25  |
| Salts IV   | 4  | Ca pantothenate     | 2.0   |
| Vitamins A, D, E and K supplied as haliver oil fortified with $\alpha$ -tocopherol and 2 methyl naphthoquinone |    | Choline chloride    | 100.0 |
|  |    | Niacin              | 5.0   |
|  |    | Inositol            | 10.0  |
|  |    | Biotin              | 0.01  |
|  |    | Folic acid          | 0.02  |
|  |    | p Aminobenzoic acid | 25.0  |

TABLE 2 EFFECT OF PHTHALYLSULFATHIAZOLE ON THE FOLIC ACID CONTENT OF LIVER AND CECUM OF RATS

|  | SUCROSE<br>BASAL | SUCROSE<br>BASAL + 2%<br>DRUG |
|--|------------------|-------------------------------|
| Average growth/week for 4 wks                                  | 31               | 12                            |
| Folic acid content of liver, $\gamma$ /gm                      | 0.7              | 0.3                           |
| Folic acid content of cecal contents after 2 wks, $\gamma$ /gm | 0.85             | 0.03                          |

when the rats are kept in a tube cage than when kept in the ordinary cage. A definite modification in the composition of the flora in the cecum can be demonstrated during these changes (21). The drug depresses both the coliform and lactobacillus groups. The coliform group is changed most rapidly but shows a tendency toward reestablishment. The lactobacillus group is altered more slowly but the change is more permanent.

The mouse and the dog are similar to the rat in that no folic acid is needed preformed in the diet. However, the chick and the monkey require definite amounts although the type of carbo-

hydrate and the level of fat may influence the total amount needed by the chick (22). Succinylsulfathiazole does not produce a more severe folic acid deficiency in the monkey, but it may increase the requirement for the chick (23).

Very similar results are available for biotin. The addition of succinylsulfathiazole precipitates a biotin deficiency in the rat and Wright and Welch (24) have reported a decrease in the liver biotin from 0.7 to less than 0.3  $\gamma$ /gram of fresh tissue as the deficiency develops. While growing chicks must receive a definite supply of preformed biotin in the ration, laying hens can obtain appreciable quantities through intestinal synthesis when the basal ration contains dextrin. Data in table 3 obtained by Couch *et al.* (25) show that the biotin content of eggs, from hens receiving dextrin as the

TABLE 3 BIOTIN CONTENT OF EGGS FROM HENS FED DIFFERENT CARBOHYDRATES

| RATION           | 0 WKS                         | 3 WKS | 8 WKS |
|------------------|-------------------------------|-------|-------|
|                  | $\gamma$ /gram fresh material |       |       |
| Sucrose          |                               |       |       |
| Yolk             | 0.5                           | 0.05  | 0.01  |
| White            | 0.1                           | 0.00  | 0.00  |
| Dextrin          |                               |       |       |
| Yolk             | 0.5                           | 0.35  | 0.15  |
| White            | 0.1                           | 0.02  | 0.004 |
| Practical        |                               |       |       |
| Yolk             | 0.5                           | 0.50  | 0.5   |
| White            | 0.1                           | 0.10  | 0.1   |
| Sucrose + biotin |                               |       |       |
| Yolk             | 0.5                           | 0.60  | 0.5   |
| White            | 0.1                           | 0.11  | 0.11  |

carbohydrate, is much higher than that of eggs from hens on sucrose rations. The values do not equal those obtained for eggs from hens on practical rations but the level is high enough to allow good hatchability. The eggs from the hens on the sucrose ration fail to hatch after two weeks while eggs from hens receiving the dextrin ration continue to hatch over a period of eight months. Johansson, Shapiro and Sailes (26) found that the feces from the hens on the sucrose ration were nearly devoid of coliform organisms and that yeast largely replaced the coliform flora. Feces from the hens receiving dextrin contained 10 times the number of coliforms found in the feces from hens receiving sucrose plus biotin. Sama, Snell and Elvehjem (27) have presented definite evidence for an increased synthesis and utilization of vitamin B<sub>6</sub> when dextrin is used in place of sucrose in purified rations.

Cunha *et al.* (28) found no beneficial effect on external appearance, growth or efficiency of feed utilization in pigs during a seven-week period when either folic acid or para-aminobenzoic acid was added alone or in combination with inositol and biotin to the basal ration containing the first six B vitamins listed in table 1. However, Lindley and Cunha (29) found the pig did need biotin or inositol when phthalylsulfathiazole was included at a level of 0.5 per cent in the ration. The addition of inositol alone alleviated to a large extent the symptoms prevented by biotin, which indicates that inositol may stimulate the synthesis of this vitamin. Woolley (30) has presented the best evidence for the bacterial synthesis of inositol. Organisms, cultivated from the intestinal tract of mice which had exhibited a spontaneous cure of inositol deficiency, synthesized this vitamin to a much greater extent than did organisms isolated from the tract of deficient mice. The organism was shown to be a gram-negative one but not the most prominent gram-negative organism in the tract, namely *E. coli*, because it does not form inositol. Spitzer and Phillips (31) have reported that rats fed certain soybean oil meal rations develop a characteristic alopecia which responds to either inositol or biotin. Furthermore, cystine or methionine were effective and they suggest alterations in the intestinal flora as one of the possible mechanisms involved in these relationships.

There is ample evidence for the synthesis of niacin in the rat. On a typical synthetic ration without added niacin the excretion may be 100 times the intake (32). This undoubtedly explains the difficulty encountered by early workers in producing a pellagra-like syndrome in the rat. However, a condition which will respond to niacin can be produced in this animal by using a ration low in tryptophane and high in certain other amino acids (33), especially threonine and phenylalanine. A few typical results are given in table 4. It appears that 1.5 mgm of niacin has the same effect as 50 mgm of tryptophane, if the basal ration contains 100 mgm of tryptophane.

It is clearly established that tryptophane can be converted into niacin in the animal body, but whether this relationship is dependent upon intestinal synthesis has not been clearly established. One possible explanation is that when ample quantities of niacin are synthesized by the bacteria, part of the tryptophane does not need to be converted to niacin and is, therefore, conserved for use as an amino acid. On the other hand, a

high intake of niacin may alter the flora so that less tryptophane is destroyed by the microflora.

An argument against the action of the bacteria is that the relative requirement of these nutrients is not influenced by sulfa drugs. This fact may not be too significant because we know that the feeding of these drugs does not completely eliminate any group of organisms and, furthermore, these drugs may alter the quantity of organisms involved in the production of folic acid and biotin, but not those involved in the production or destruction of niacin. We may also mention that niacin is synthesized in the egg during incubation when no bacteria are present (34).

The best evidence in favor of bacterial activity is the fact that dextrin, high fat (35) and a low intake of certain free amino acids favor the production of niacin. Very recently Henderson and Hankes (unpublished data) have observed an

had a higher amount of cecal contents and bacterial count per cecum. No great variation in the composition of the flora was found although a gram-negative anaerobe rod occurred more frequently in the mice with the lower requirement. Excess folic acid in the diet increased the niacin synthesis and this effect was eliminated by the sulfa drug. Work with dogs (36) has shown that folic acid improves the response of these animals to standard doses of niacin.

Rosen, Huff and Perlzweig (37) found the omission of pyridoxine from the rats resulted in a progressive decrease in the urinary excretion of niacin derivatives after doses of tryptophane. This defect was not restored to normal even after two weeks of vitamin B<sub>6</sub> supplementation, and they suggested that delayed changes in the bacterial flora may account for this effect. However, they countered this suggestion with the fact that formation of these derivatives responds very rapidly to parenteral injection of tryptophane.

Several years ago du Vigneaud and coworkers (39) pointed out that an occasional animal showed some growth on a diet containing homocystine with no added cystine and suggested that this response might be due to refection. Later these workers (40) reported small but significant amounts of labile methyl groups synthesized in the rat and suggested that intestinal bacteria were involved. Thus we can find evidence for intestinal synthesis of practically all the B vitamins listed in table 1 but the degree of synthesis and utilization of the factors produced need much more study. The newer results are in agreement with the early data, namely that more synthesis usually occurs on a dextrin diet. This appears to hold for niacin, vitamin B<sub>6</sub>, biotin, folic acid and possibly unknown factors, although I doubt that we can make a general conclusion. Coates *et al* (41) clearly established the production of riboflavin during refection and that more riboflavin may be produced than thiamine. Mannering *et al* (42) found a large production and utilization of riboflavin in rats fed a ration high in lactose, but the growth was not as great as on sucrose rations. Baumann and Clayton (unpublished data) have obtained interesting results in this connection during their study on the effect of different carbohydrates on the incidence of liver tumors in rats given azo dyes. More riboflavin was found in the liver, feces and urine from rats receiving lactose than those given sucrose. With dextrin diets, the liver and feces contained a higher amount than with sucrose diets, but the urine was significantly

TABLE 4 GROWTH DEPRESSING EFFECT OF SINGLE AMINO ACIDS

|                           | AV WEEKLY GAIN IN GRAMS |          |
|---------------------------|-------------------------|----------|
|                           | - Niacin                | + Niacin |
| Basal                     | 11                      | 16       |
| " + 156% DL-threonine     | 2                       | 20       |
| " + 078% DL-threonine     | 2                       |          |
| " + 104% DL-phenylalanine | 5                       | 17       |
| " + 052% D-phenylalanine  | 9                       |          |
| " + 2% glycine            | 5                       | 16       |
| " + 2% DL-alanine         | 13                      | 14       |

actual increase in the nicotinic acid content of the feces from rats fed extra threonine, in spite of the fact that these rats suffer from a niacin or tryptophane deficiency. This change may be due to the reduced amount of feces, but the flora may be altered so that more niacin is tied up by the organisms in the lower part of the tract making less available for absorption. Teply *et al* (20) found the largest amount of niacin per gram of cecal contents of rats fed a dextrin diet. The inclusion of 2 per cent phthalysulfathiazole caused a marked decrease in the niacin on a per gram basis, but the ceca increased in size so that there was practically no change in the total amount present. It is interesting to point out in relation to these studies that Gall, Fenton and Cowgill (38), working with two strains of mice differing in their riboflavin and pantothenic acid requirements, found that the mice with the lower requirement

lower. Thus dextrin may actually allow the increase in growth of organisms which prevent the utilization of riboflavin by the animal.

Gall, Illingworth, Cowgill and Fenton (43) found interesting differences between cocci found in ceca of mice fed different carbohydrates. The coccus characteristic of the flora found in mice fed dextrin diets grew well on synthetic broth lacking folic acid and liberated large amounts of this vitamin into the environment. On the other hand, the coccus found in the animals receiving dextrose diets showed little or no growth in the folic acid-deficient broth and liberated little if any of the vitamin.

High fat rations are known to decrease the production of riboflavin, but in the presence of an adequate dietary source of this vitamin differences in the rate of growth of rats can be observed when different fats are used in lactose and sucrose rations. Differences between corn oil and butter fat (44) may be explained on the basis of an additional factor in butter fat which is limiting in these rations or on the basis that corn oil inhibits or stimulates the growth of certain organisms which produce or destroy additional factors. Nath (unpublished data) has clearly demonstrated a large decrease in the cecal coliform count when 28 per cent of fat is used in place of 10 per cent fat. Thus, if the animal depends upon the coliform organisms for a supply of certain limiting factors, it is not surprising to find deficiencies on high fat rations which are not observed on low fat rations. So far we have been unable to show consistently any differences in the flora in the rats given 28 per cent butter fat and 28 per cent corn oil. However, the variation may occur in the types which are counted with difficulty. Early in the use of microbiological assays for vitamins it was observed that fatty acids markedly affected the growth of lactic acid bacteria. Williams and Fieger (45) found that oleic acid can eliminate the requirement of *L. casei* for biotin. Williams, Broquist and Snell (46) found oleic acid essential for certain cultures of lactic acid bacteria even in the presence of biotin, but for a strain of *L. bulgaricus* oleic acid was found to be so toxic that its growth-promoting action can only be observed within a very narrow range of concentration. These few examples can readily explain why fats may have a profound effect on the intestinal flora.

In guinea pigs, Booth (unpublished data) has recently shown that there is little advantage of dextrin over sucrose in the synthetic rations he has used (table 5). However, the addition of gum arabic or other pentosan-rich materials produces a

significant improvement in the growth and appearance of these animals. The effect may be related to an increased synthesis of certain factors or it may tend to decrease the number of unfavorable organisms. Since liver and other foods high in unknown factors do not produce a beneficial effect, the latter explanation may be a possibility. Hale, Duncan and Hoffman (47) have recently shown that iodophile microorganisms in the rumen play an important role in digestion and, furthermore, function in the synthesis of fatty acids when alfalfa hay or beet pulp rations are fed. Both of these materials have been found beneficial in the guinea pig. Reyniers (2) has presented evidence that germ-free guinea pigs fail to utilize either purified or crude rations although they eat readily and he suggests that

TABLE 5 GROWTH OF GUINEA PIGS ON PURIFIED RATIONS

| RATION                                     | NO OF ANIMALS | MORTALITY | GAIN IN WEIGHT<br>gm/day |
|--|---------------|-----------|--------------------------|
| 1 Basal sucrose                            | 9             | 2         | 2.4                      |
| 2 Basal dextrin                            | 7             | 4         | 1.9                      |
| 3 Basal sucrose + gum arabic               | 12            | 0         | 3.8                      |
| 4 Basal dextrin + gum arabic               | 6             | 1         | 4.0                      |
| 5 Basal sucrose + gum arabic + 15% alfalfa | 6             | 0         | 6.6                      |
| 6 Stock ration                             | 27            | 0         | 7.1                      |

All results calculated after 6 weeks. Animals on rations 3 and 4 failed after six weeks.

digestion may be impaired in the absence of the microorganisms.

Although Silmon (9) emphasized that the consumption of feces was more important when natural foods were fed, there is evidence that the requirement for unknown factors may be greater when natural food mixtures are used. If we return to table 1, we find that rats grow 25-30 grams per week on this synthetic ration, and the rate can be increased 6-8 grams per week by supplementing the ration with liver preparations. More uniform differences are obtained if a corn-soybean ration is used in place of the synthetic mixture (48). The results are even more striking when chicks are used (49), and a few typical results are given in table 6. Part of the difference between the two diets may be due to the presence of casein, since Cary and coworkers (50) have shown that casein

carries an additional growth factor for rats. However, our chick rations contain 7.5 per cent casein and a significant growth response to liver fractions is still obtained. Evidence for the bacterial synthesis of this factor has been obtained by Rubin, Burd and Rothchild (51) who find a factor present in the feces of hens.

When a greater variety of processed foods is used for rats a growth retardation is obtained which is counteracted only upon the addition of both extra amounts of B vitamins and casein or methionine (table 7). Since these original rations contain sufficient B vitamins to meet the ordinary requirements of the rat, this effect may also be dependent upon changes in the micro flora. The relation of vitamins and amino acids is not surprising since we are finding more and more evi-

ever, if niacin is omitted and no folic acid is supplied, a condition is finally obtained which responds to the same liver fractions which are active in the chick. Another interesting condition is obtained if dogs are maintained on the synthetic diet without folic acid and with no added fat. The animals show a rapid decline in weight and they can only be saved by giving folic acid plus butter fat. Either one alone is not effective and folic acid plus corn oil does not work. However, folic acid plus a mixture of adenine, thymine and uracil is active. The kind of fatty acid present may be important along with folic acid in the synthesis of purines.

So far no mention has been made of the ability of different species of bacteria to synthesize vitamins and amino acids, the degree of synthesis or the distribution of the compounds formed between the cells and the surrounding medium.

TABLE 6 GROWTH RESPONSE IN CHICKS FED CORN-SOYBEAN MEAL RATIONS

|  | AV. WT. 4 WEEKS |
|--|-----------------|
|  | gm              |
| Basal  | 159             |
| " + 3% Condensed fish solubles                             | 259             |
| " + .05 cc Reticulogen per chick per day (1 U S P unit)    | 240             |
| " + .005 cc Reticulogen per chick per day (1 U S P unit)   | 254             |
| " + .001 cc Reticulogen per chick per day (.02 U S P unit) | 210             |
| " + .065 cc Armour prep per chick per day (1 U S P unit)   | 228             |

TABLE 7 GROWTH OF RATS ON A MIXTURE OF PROCESSED FOODS

|   | AV. WT. AT END OF 5 WEEKS |
|---|---------------------------|
|   | gm                        |
| Basal   | 112                       |
| Basal + all 'B' vitamins                      | 144                       |
| Basal + 5% casein                             | 158                       |
| Basal + all 'B' vitamins + 5% casein          | 192                       |
| Basal + all 'B' vitamins + 0.6% DL-methionine | 184                       |

dence for interrelationships between these substances in metabolisms. We need only mention the relation of biotin to aspartic acid (52), tryptophane to niacin and vitamin B<sub>6</sub>, and the recent work of Kidder and Dewey (53) that serine can act as an antagonist to the growth inhibition produced in the silicated protozoa by nine essential amino acids. In this connection I might mention that the action of certain vitamin antagonists may be produced through their effect on intestinal bacteria as well as on the animal tissues. For example Banejee and Elvehjem (54) found the unfavorable effect of glucoascorbic acid in rats was counteracted by liver extract rather than ascorbic acid.

The effect of the level of B vitamins in the diet on an animal's requirement for other factors is best exemplified in recent work with dogs. Dogs grow and develop remarkably well on synthetic diets containing the first six B vitamins. How-

Results available up to 1945 have been reviewed by Peterson and Peterson (55). The importance of secretion of vitamins into the medium was emphasized by Thompson (56) who showed that the liberation of biotin by *Proteus vulgaris* parallels the growth or may even precede growth. Mitchell and Isbell (57) have concluded that inositol, nicotinic acid, riboflavin and thiamine are found to a considerable extent within the cells and appear in the surrounding medium to a small extent, that pantothenic acid and folic acid diffuse to a somewhat larger extent and that biotin and pyridoxine apparently move freely from the cells into the medium. The opposite reaction is certainly a factor in nutrition since Ness, Price and Parsons (58) have found that the administration of fresh yeast to human subjects reduces the absorption of thiamine by the body. The avidity with which microorganisms take up vitamins apparently depends upon whether the vitamin in question is an essential nutrient or can be

produced by the cells themselves Chang and Peterson (unpublished data) have shown that *Saccharomyces cerevisiae* will take up very large quantities of biotin when it is added to the medium (200 times that on a low biotin medium), while *Torula utilis* which can make its own biotin takes up very little Further, Krueger and Peterson (59) have shown that *Lactobacillus pentosus* 124-2, which needs biotin, takes up very large quantities from the culture medium In the digestive tract, therefore, we are dealing with variations in the synthetic ability of different organisms, with variations in the liberation of the vitamins from the cell and variations in the hoarding ability of neighboring cells

Time does not permit a discussion of the significance of all these interrelationships in human nutrition Perhaps it is only necessary to mention

the recent work of Denko *et al* (60), which indicates that intestinal bacteria can synthesize large quantities of certain B vitamins when healthy young adults are maintained on a restricted intake of these vitamins The absorption from the tract may be the limiting factor, since the urinary excretion decreased during reduced intake in all cases except biotin and pantothenic acid, while the fecal excretion showed little change If we disregard for a moment the complicating factor of absorption figures showing total excretion in human subjects, daily requirement and synthetic ability of different organisms can be tabulated for several B vitamins Such figures are given in table 8 It is evident that there is a rough correlation between the synthetic ability of the bacteria and the total excretion for some of the vitamins However, we must not place too much emphasis on this correlation because the organisms showing highest synthetic ability may not flourish in the tract The fact that under optimum conditions a significant fraction of the total requirement of a known vitamin may originate from the intestinal flora is not nearly as important in human nutrition as the possibility that under

disturbed conditions the supply of an unknown factor which is normally produced by the flora is reduced to a point where a conditioned deficiency develops This is the area of nutrition which needs greatest attention and which will yield the most valuable results in the next few years

In summary, I doubt that anyone can question the importance of intestinal flora in nutrition In fact, it is surprising that so little attention has been given to this subject during the evolution of our knowledge Certainly the quantitative requirement of many of the vitamins, some of the amino

TABLE 8 SYNTHESIS OF VITAMINS BY BACTERIA AND THE EXCRETION OF VITAMINS BY HUMAN SUBJECTS

|                        | CONTENT/CELL<br>AND CULTURE<br>FILTRATE | DAILY<br>HUMAN<br>REQUIRE<br>MENT | ML. NECES-<br>SARY TO<br>SUPPLY<br>REQUIRE<br>MENT | EXCRE-<br>TION<br>INTAKE |
|------------------------|---|-----------------------------------|--|--------------------------|
|                        | $\gamma/ml$                             | $mgm$                             |  |                          |
| Thiamine               | 0032- 15                                | 1 5                               | 10,000   | 1                        |
| Riboflavin             | 02 -8 5                                 | 2 0                               | 250  | 3                        |
| Niacin                 | 028 -4 6                                | 15 0                              | 3,000  | 1                        |
| Vitamin B <sub>6</sub> | 0019- 024                               | 1 5                               | 60,000   | $\frac{1}{2}$            |
| Pantothenic<br>acid    | 0303-0 99                               | 5 0                               | 5,000  | 3                        |
| Biotin                 | 0005- 035                               | 0 1                               | 3,000  | 8                        |

acids and perhaps some of the fatty acids are directly dependent upon the intestinal bacteria, and indirectly the microflora is probably related to the requirement of practically all nutrients It is unnecessary to emphasize that the data which I have summarized are far from complete and that much more work is needed However, the proper interpretation of the results which are now available and the additional studies carried on in the future will not only be valuable in nutrition but will be useful in understanding the fundamental metabolism of all living cells

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# RENAL EXCRETION OF ACID

ROBERT F PITTS

*From the Department of Physiology, Syracuse University College of Medicine*

In the metabolism of proteins and lipids, organically bound phosphorus, sulfur and chlorine are liberated as strong acid. This acid is immediately neutralized by base derived from the several body buffers. Were the acid excreted in combination with fixed base, body reserves would be rapidly exhausted, for the total store of available alkali is approximately one mol. To avoid depletion of these limited reserves, the kidneys excrete acid, in part in free titratable form and in part combined with ammonia.

Under ordinary circumstances the production of fixed acid exceeds the intake of available base by 50 to 100 milliequivalents per day. However, following the ingestion of alkali, the net acid load may decrease to zero, while in severe ketosis it may increase to as much as 750 milliequivalents in 24 hours. When renal function is normal, variations in acid load are nicely compensated by variations in titratable acid and ammonia excretion. When renal function fails, acidosis rapidly supervenes.

In the experiment described in figure 1, a normal subject was maintained for 15 days on a diet constant with respect to calories, protein and electrolyte. Successive 24-hour urine samples were collected and analyzed for selected ionic constituents. The rates of excretion of these several constituents, expressed in milliequivalents per day, are plotted in block form. During the first 5 days, which constitute the control phase of the experiment, the net endogenous acid load averaged 79 milliequivalents per day, 35 milliequivalents of which were excreted in combination with ammonia and 44 milliequivalents as free titratable acid. The subject was in electrolyte balance, excreting on an average 140 milliequivalents of sodium, 130 milliequivalents of chloride and 41 millimols of phosphate per day. In this chart the excretion of sodium is plotted downward from that of ammonia and acid to emphasize its opposite relation to base economy.

The acid load on the body was increased sharply during the second five-day period by the ingestion of 10 to 15 grams of ammonium chloride per day. This salt, immediately upon its absorption into the body, is converted into urea and hydrochloric acid. Fifteen grams of ammo-

nium chloride are, therefore, equivalent to 280 cc. of normal hydrochloric acid.

It is evident that chloride excretion rose sharply on the first day of increased acid load. Urine pH dropped from a mean control level of 5.7 to 4.9, but since little hydrochloric acid can exist free in urine of this reaction, and since ammonia excretion was only moderately increased, the excess chloride was almost entirely neutralized by sodium drawn from the buffer stores of the body.

During the subsequent four days of acid ingestion this threat to the alkali reserve was met by the excretion of more acid urine, but, most significantly, by the excretion of increased quantities of ammonia. As ammonia excretion increased, base loss diminished in proportion, until on the last day of acid ingestion a positive sodium balance was attained. Recovery had thus begun although the acid load was still great.

The processes of recovery are well illustrated in the final five-day period of this experiment. On the first day of recovery essentially no sodium was excreted, all of that ingested in the diet was retained. Anions were largely eliminated in combination with ammonia, to a lesser extent as free titratable acid. Retention of base was evident for five days, during which time the excess lost in the period of acidosis was restored to the body in full. It should be emphasized that the ingestion of ammonium chloride gives a rather warped view of the processes of base economy for it reduces to relative insignificance the excretion of titratable acid. Hydrochloric acid is a strong acid and relatively little can be excreted free in urine of maximal acidity. Had the acid been a weaker buffer acid, such as beta-hydroxybutyric, a relatively greater contribution of titratable acid excretion would have been evident.

These results which were obtained by Drs. Sartorius and Roemmelt (1) in the course of a study of renal function in acidosis are not in themselves new, having been described previously by Drs. L. J. Henderson, Gamble, Van Slyke and others who have contributed so significantly to an understanding of acid-base metabolism. However, they serve well to illustrate the metabolic significance of two renal mechanisms which I wish to discuss, namely the mechanism of produc-



tion of acid urine and the mechanism of secretion of ammonia

Let us begin by considering the mechanism which transforms the slightly alkaline blood plasma into acid urine. The studies of Montgomery and Pierce (2) on the amphibian kidney provide a morphological basis for an understanding of this process. These investigators withdrew minute quantities of fluid from the glomerulus and from the proximal, intermediate, and distal

of the urine is thus a function of the distal segment of the renal tubule.

There are, obviously, two general means by which the distal tubule could convert its slightly alkaline contents into acid urine: first, it might reabsorb the alkaline components of the buffer mixture which enters the glomerular filtrate, leaving an acid residue to be discharged into the urine; second, it might add acid to the tubular contents. Four of the several possible permuta-

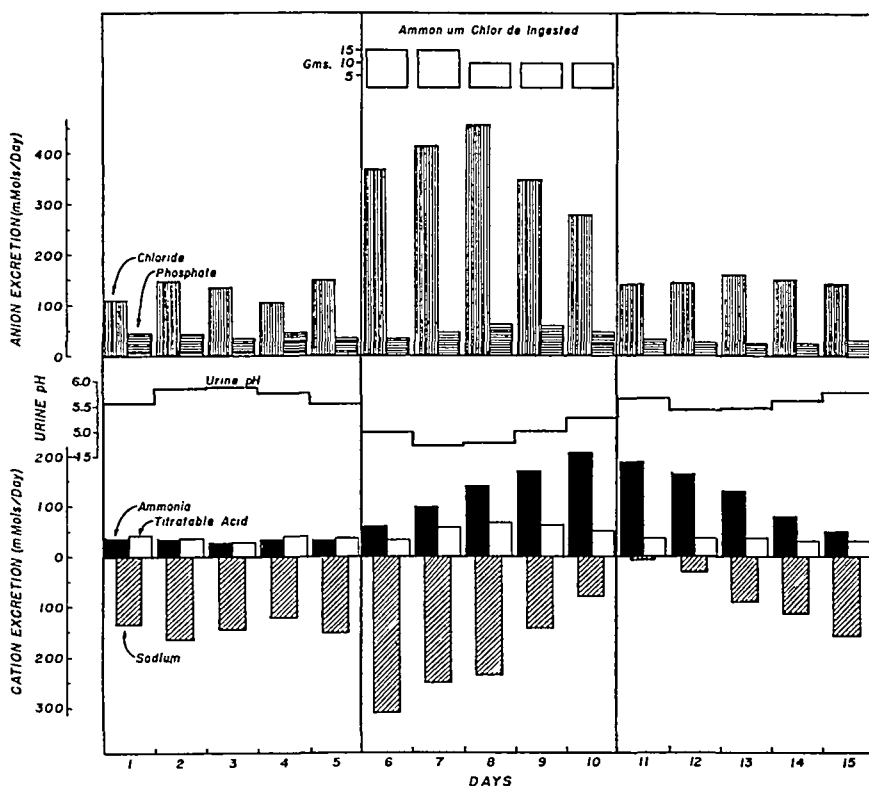


FIG 1 Renal excretion of ions in ammonium chloride acidosis (1)

segments of the nephron and determined the pH with a micro quinhydrone electrode. Figure 2, taken from their work, compares the reaction of the fluid obtained at each level with that of the plasma from which it was formed. It is evident that within reasonable limits the pH of the glomerular filtrate and the tubular fluid obtained from the proximal and intermediate segments is identical with that of the plasma. In the distal tubule the urine becomes acid, the pH decreasing to values as low as those observed in urine obtained from the ureter and bladder. Acidification

of these two basic hypotheses are described in figure 3 (3).

Only two buffer mixtures enter the glomerular filtrate in significant quantities, namely monobasic and dibasic phosphate, and carbonic acid and bicarbonate. If, as shown in the upper diagram of the nephron, dibasic phosphate were preferentially reabsorbed, the excreted monobasic phosphate could be titrated as acid in the urine. This concept, which may reasonably be termed the *phosphate reabsorption theory*, appears in a number of current Biochemistry texts. On the

other hand, if bicarbonate were completely reabsorbed, and if the tubule were impermeable to carbonic acid, as claimed by Sendroy, Seelig and Van Slyke (4) in their *bicarbonate reabsorption theory*, this acid would react with buffer salts to convert them quantitatively into free buffer acid.

Similarly two hypothetical mechanisms depending on active tubular transfer have been proposed as explanations of urinary acidification. The *tubular secretion of molecular acid* has been invoked by Macallum and Campbell (5) to account for the conversion of alkaline buffer components into their acid forms. On the other hand, Homer Smith (6) has proposed an *ionic exchange mechanism* which would accomplish the

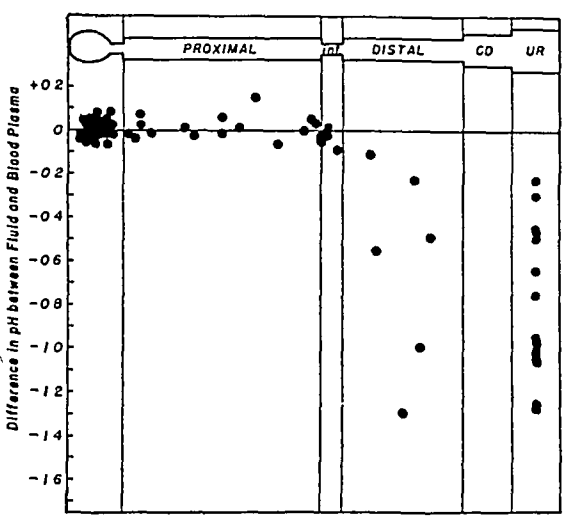


FIG. 2 Relationship between the reaction of the tubular urine and that of the plasma at various levels in the amphibian nephron (2)

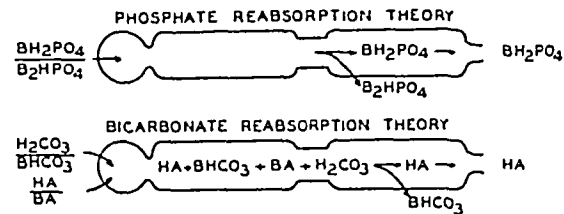
same end. According to this latter view, hydrogen ions, formed within the tubular cells by the dissociation of carbonic acid, are exchanged for ions of fixed base bound by buffer salts in the tubular urine, thereby converting them into titratable buffer acids. To describe these latter two mechanisms as secretory is only partially justified, for reabsorption obviously plays a significant rôle in each.

It occurred to Dr. Alexander and to me (7) that it should be possible to test experimentally these several theories, for each has inherent within it a specific identifiable limitation of its capacity to cause the excretion of acid. From our studies on acidotic dogs, we concluded that the ionic exchange concept provides the most adequate explanation of the mechanism involved.

I shall illustrate the principles involved in test-

ing these several hypotheses with some experiments recently performed by Drs. Ayer, Lot-speich, Schiess and myself (3) on man, for just as conclusive results can be obtained in man as in the dog. As the subject of the experiment summarized in table 1, I ingested 20 grams of ammonium chloride on the preceding day to produce a moderately severe acidosis. The extent of the acidosis is illustrated by the low bicarbonate content of arterial plasma, a value of 14.8 milli-

URINARY ACID PRESENT IN ORIGINAL FILTRATE



URINARY ACID SECRETED INTO TUBULAR URINE

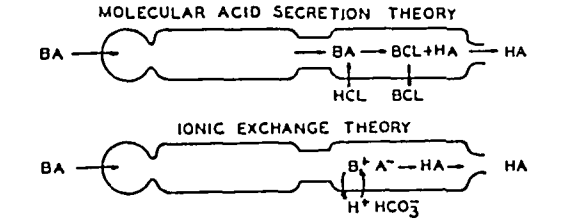


FIG. 3 Theories to account for the excretion of acid urine (3)

TABLE 1 AN EXPERIMENT ON A NORMAL HUMAN SUBJECT IN EXPERIMENTAL ACIDOSIS DESIGNED TO TEST CRITICALLY THE SEVERAL THEORIES OF URINARY ACIDIFICATION

| GLOW FILT. RATE | PLASMA            |                                |      | PHOSPHATE   |            |            | H <sub>2</sub> CO <sub>3</sub> |       | URINE             |         |
|-----------------|-------------------|--------------------------------|------|-------------|------------|------------|--------------------------------|-------|-------------------|---------|
|                 | BHCO <sub>3</sub> | H <sub>2</sub> CO <sub>3</sub> | pH   | FILTR. ERD. | EXCR. ETED | REABSORBED | FILTR. ED                      | pH    | TITRAT. ABLE ACID | mE./mL. |
| 102             | 14.8              | 0.86                           | 7.34 | 5.45        | 0.556      | 0.419      | 0.137                          | 0.087 | 4.64              | 0.328   |
| 101             | 14.8              | 0.86                           | 7.34 | 6.04        | 0.610      | 0.455      | 0.155                          | 0.087 | 4.63              | 0.348   |
| 98.7            | 14.6              | 0.82                           | 7.35 | 6.49        | 0.640      | 0.86       | 0.154                          | 0.081 | 4.60              | 0.371   |
| 100.            | 14.8              | 0.83                           | 7.35 | 6.73        | 0.673      | 0.516      | 0.157                          | 0.083 | 4.56              | 0.395   |

moles per liter being roughly half the normal. The low carbonic acid content and the significantly reduced pH of the plasma are likewise indicative of moderately severe yet fairly well compensated acidosis. Sodium thiosulfate was infused in order that its clearance could be used as a measure of glomerular filtration rate. Neutral sodium phosphate was infused to elevate the plasma phosphate concentration to a value some five to six times the normal, in order to provide the kidney with large quantities of buffer substrate upon

which to operate Four clearance periods were then performed From the data of the first five columns of this table one may calculate the quantities of phosphate filtered, excreted and reabsorbed, and the quantity of carbonic acid filtered You observe from the data presented in the last two columns that the urine formed in each of the four clearance periods was highly acid, the pH averaging about 4.6 Between 0.3 and 0.4 milliequivalent of titratable acid was eliminated per minute This is equivalent to the excretion of 5 to 6 liters of 0.1 N acid per day, a quantity some three to four times the highest ever observed in severe diabetic ketosis This high rate of excretion of titratable acid is the direct consequence of the high rate of excretion of a buffer of nearly ideal acid strength

TABLE 2 CRITICAL ANALYSIS OF THE DATA FROM TABLE 1 WHICH INDICATES THE INADEQUACY OF THE PHOSPHATE AND CARBONIC ACID EXPLANATIONS OF URINARY ACIDIFICATION

| URINARY TITRATABLE ACID |                    |                  |                               |                  |                                 |                  |
|-------------------------|--------------------|------------------|-------------------------------|------------------|---------------------------------|------------------|
| OBSERVED                | CALCULATED FROM    |                  |                               |                  |                                 |                  |
|                         | PHOSPHATE EXCRETED |                  | PHOSPHATE REABSORPTION THEORY |                  | BICARBONATE REABSORPTION THEORY |                  |
|                         | mEq/min.           | percent observed | mEq/min.                      | percent observed | mEq/min.                        | percent observed |
| 0.328                   | 0.322              | 98.2             | 0.031                         | 9.5              | 0.087                           | 26.5             |
| 0.348                   | 0.350              | 100.5            | 0.035                         | 10.0             | 0.087                           | 25.0             |
| 0.371                   | 0.376              | 101.4            | 0.034                         | 9.2              | 0.081                           | 21.8             |
| <u>0.395</u>            | <u>0.399</u>       | <u>101.1</u>     | <u>0.035</u>                  | <u>8.9</u>       | <u>0.083</u>                    | <u>21.0</u>      |
| 0.361                   | 0.362              | 100.3            | 0.034                         | 9.4              | 0.085                           | 23.6             |

From these same data it is possible to calculate the rate of excretion of titratable acid in three ways first, from the measured excretion of phosphate and pH of the urine, second, from the measured rate of reabsorption of phosphate, granting the underlying premises of the phosphate reabsorption theory, and third, from the measured rate of filtration of carbonic acid, conceding the assumptions of the bicarbonate reabsorption theory The values calculated in these three ways are expressed in table 2 both in absolute units and in percentage of the observed values Values calculated from phosphate excreted should agree closely with observed values for they merely provide a check on the accuracy of the chemical determinations Agreement within limits of 98.2 and 101.4 per cent is more than adequate for our purposes

The significant features of these calculations are apparent in the last four columns I call your attention to the fact that the phosphate reabsorption theory can explain only 8.9 to 10 per cent of the observed excretion of acid and that the bicarbonate reabsorption theory can explain only 21 to 26.5 per cent of the observed acid Under the conditions of our experiments, these two theories together can account at a maximum for only one third of the acid actually eliminated

Four similar experiments on the four collaborators in this investigation are briefly summarized in table 3 The general inadequacy of both the phosphate reabsorption theory and the bicarbonate reabsorption theory, in explaining the excretion of acid, is evident Since carbonic acid and monobasic phosphate are the only acids present in the glomerular filtrate in significant

TABLE 3 SUMMARY OF EXPERIMENTS ON 4 ACIDOTIC SUBJECTS ANALYZED ACCORDING TO THE METHOD OUTLINED IN TABLE 2

| URINARY TITRATABLE ACID |          |                    |                  |                               |                  |                                 |                  |
|-------------------------|----------|--------------------|------------------|-------------------------------|------------------|---------------------------------|------------------|
| SUBJECT                 | OBSERVED | CALCULATED FROM    |                  |                               |                  |                                 |                  |
|                         |          | PHOSPHATE EXCRETED |                  | PHOSPHATE REABSORPTION THEORY |                  | BICARBONATE REABSORPTION THEORY |                  |
|                         |          | mEq/min.           | percent observed | mEq/min.                      | percent observed | mEq/min.                        | percent observed |
| R.F.P.                  | 0.361    | 0.362              | 100.3            | 0.034                         | 9.4              | 0.085                           | 23.6             |
| WAS.                    | 0.386    | 0.402              | 104.1            | 0.031                         | 8.0              | 0.104                           | 27.0             |
| J.L.A.                  | 0.351    | 0.352              | 100.2            | 0.032                         | 9.1              | 0.129                           | 36.8             |
| W.D.L.                  | 0.350    | 0.360              | 103.0            | 0.036                         | 10.3             | 0.142                           | 40.6             |

amounts, these experiments are conclusive in proving that acid must be added to the tubular urine by some active cellular mechanism <sup>1</sup>

<sup>1</sup>Theoretically the acid could be added in several ways 1) secreted as molecular acid, 2) formed by the exchange of hydrogen ions for fixed base, 3) formed de novo within the tubules from water by the reabsorption of hydroxyl ions, or 4) formed de novo from bicarbonate by the reabsorption of carbonate ions Briefly, we feel that secretion of molecular acid is unlikely because no evidence exists for renal secretion of any strong acid anion Such ions as chloride, sulfate and phosphate are reabsorbed more or less completely, not secreted We likewise feel that the formation de novo of acid within the tubule by reabsorption of hydroxyl or carbonate ions is improbable because this would require the very considerable uptake of these ions from a fluid in which their concentrations are vanishingly low The most efficient anion reabsorptive mechanism known, namely that for bicarbonate, is capable of 99.99+ per cent removal to a final concentration of 10<sup>-7</sup> molar To accomplish acidification, 5 by

Time does not permit a discussion of the reasons for our view that ionic exchange accomplishes acidification of the urine, nor for our evidence and the evidence of Wilhelm, Hoeber and others that carbonic anhydrase is an essential enzymatic link in the chain of processes by which exchange is accomplished. Rather I shall present our current view of the nature of the exchange mechanism.

Figure 4 represents a cell from that portion of the distal tubule which is concerned with acidification of the urine. Such a cell is exposed on one side to the tubular blood, on the other to the tubular urine. By virtue of its own metabolic activities, as well as its exposure to the renal capillary blood, a continuous supply of carbon dioxide is available to it. Because of its high

hydrogen ion, along with the anion residue, is excreted in the urine as titratable acid.

Obviously these transfers cannot occur spontaneously, energy must be cycled into the system, but the nature of these energy-yielding processes has not been determined. An important characteristic of the mechanism which limits its transfer capacity is known from the work of Henderson and others, namely that there is a maximum gradient against which the cell can transfer hydrogen ions, a gradient of approximately 800 to 1, that is, the urine is limited in acidity to pH 4.5, the blood being pH 7.4<sup>2</sup>.

Largely in consequence of this limitation, three factors determine the rate of exchange, and thus the rate of excretion of titratable acid: first, the quantity of buffer in the tubular urine upon which the acidifying cells may operate, second, the acid strength of this buffer, and third, the degree of acidosis (8).

The operation of these factors is illustrated in the next three charts which describe a series of experiments performed on a single normal subject. In the experiment shown in figure 5, in which a moderately severe acidosis was induced, neutral sodium phosphate was infused in progressively increasing amounts to cause the excretion of increasing quantities of buffer in the urine. It is evident that the rate of excretion of titratable acid is directly proportional to the quantity of buffer presented to the renal tubules per unit of time.

Three experiments performed on the same subject in comparable states of acidosis are summarized in figure 6. In one the weakly acidic buffer phosphate was administered, in another the moderately acidic buffer creatinine, and in the third the strongly acidic buffer para-aminohippurate. It is evident, at any given molar rate of excretion, that the kidney exchanges hydrogen ions for base more effectively the less dissociated is the resulting buffer acid, that is, the lower the hydrogen ion gradient against which transfer must be accomplished.

In two similar experiments shown in figure 7, creatinine was infused. In one experiment acid-base relationships were normal, in the other, sufficient ammonium chloride had been ingested

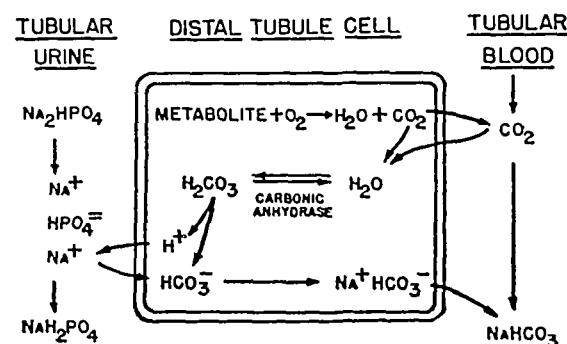


FIG 4 Nature of the cellular mechanism for acidification of the urine (7)

content of carbonic anhydrase the cell can rapidly transform this dissolved gas into carbonic acid. Hydrogen ions dissociated from carbonic acid are exchanged across the luminal border of the cell for ions of fixed base in the tubular urine. The base, along with an equivalent quantity of bicarbonate, is returned to the renal venous blood. The

hydroxyl or carbonate ion absorption would require that the efficiency of the reabsorptive mechanism be nearly 1000 times that of the bicarbonate mechanism.

It is probable that the kidney adjusts the reaction of the urinary buffer mixture by adjusting the concentration of a single ion species, the concentrations of all other ion species being thereby determined through isohydric equilibrium. It seems to us that the basic biologic significance of acidification of the urine is the restoration to the body of the fixed base present in the glomerular filtrate, and the excretion of unwanted anions in combination with hydrogen ions. Certainly these ends are most simply attained by a tubular exchange mechanism such as that proposed above.

<sup>2</sup> In reality the significant  $H^+$  ion gradient is not that from peritubular blood to tubular urine, but that from tubular cell contents to tubular urine. Since measurement of this latter gradient is impossible, we may argue from the former measurable one, at all times keeping in mind its limitations.

to reduce the plasma bicarbonate roughly by half. It is obvious, at all rates of buffer excretion, that the rate of elimination of titratable acid is greater in acidosis than when plasma bicarbonate is

tween titratable acid excretion and plasma bicarbonate concentration is illustrated in figure 8. On the left are represented the conditions which obtain in acidosis. Fair quantities of fixed buffer,

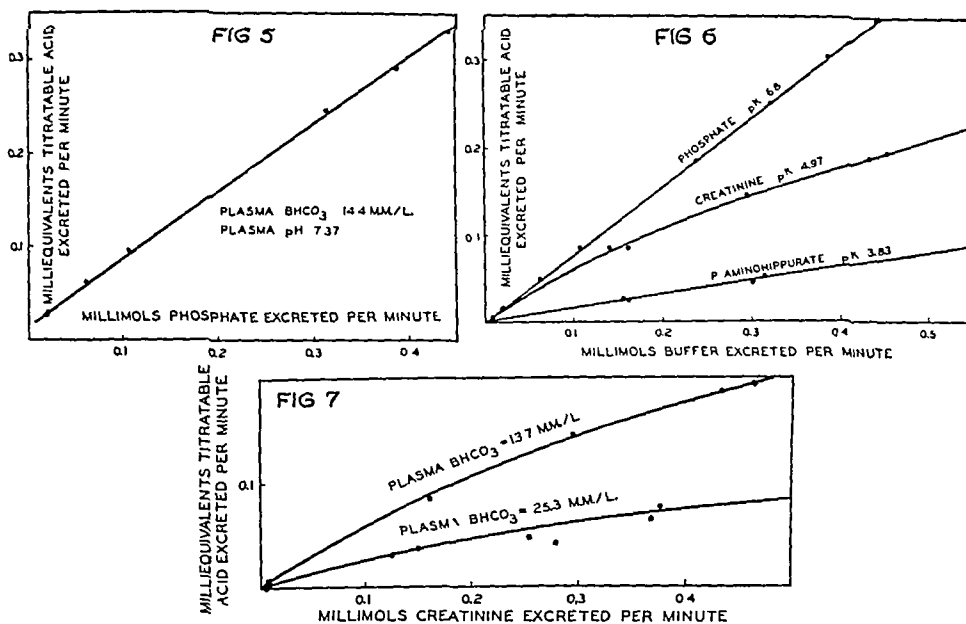


FIG 5 Relationship between the rate of excretion of titratable acid and the rate of excretion of phosphate in the normal human subject in ammonium chloride acidosis (S)

FIG 6 Relationship between the rate of excretion of titratable acid and the acid strength of the buffer in the normal human subject in ammonium chloride acidosis (S)

FIG 7 Relationship between the rate of excretion of titratable acid and the degree of depletion of the alkali reserve in the normal human subject (S)

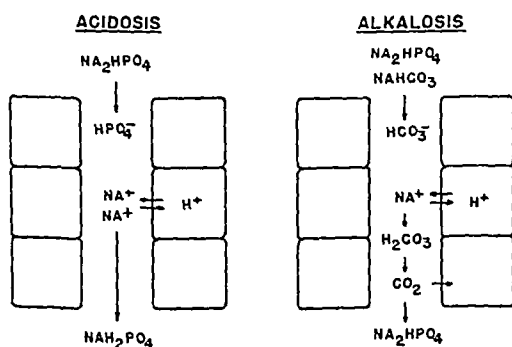


FIG 8 Diagrammatic explanation of the basis for the inverse relationship between titratable acid excretion and plasma bicarbonate concentration (14)

normal. But what is rather surprising is the high rate of excretion of acid attained in the normal state. It appears that conditions accepted as indicative of normalcy constitute a state of mild acidosis so far as the kidneys are concerned.

Our concept of this inverse relationship be-

but little bicarbonate, enter the distal segment of the renal tubule. The exchange of hydrogen ions for base, bound by the buffer, proceeds unopposed. On the right are represented the conditions which obtain normally and which are exaggerated greatly in alkalosis. In consequence of elevated plasma level and increased delivery into the glomerular filtrate, bicarbonate as well as fixed buffer enters the distal segment. The two compete as donors of base with the result that the excretion of titratable acid is reduced (9).

To Nash and Benedict belongs the credit for first demonstrating that the tubular cells form ammonia from some precursor in the arterial blood and actively secrete it in high concentration into the tubular urine. In figure 9 are summarized experiments of Walker (10) which show that the secretion of ammonia, like the elaboration of acid urine, is a function of the distal tubule. Fluid drawn from the glomerulus and proximal tubule of the amphibian kidney contains only an insignificant trace of ammonia. As fluid traverses the

distal segment, ammonia is added in increasing amounts

In the past there has been disagreement concerning the nature of the plasma precursors of ammonia, although a majority have accepted urea as the probable source. Recently Archibald demonstrated the presence of glutamine in the circulating blood plasma, and of the enzyme glutaminase in the kidney. Following up these observations, the group (11) working in Dr. Van

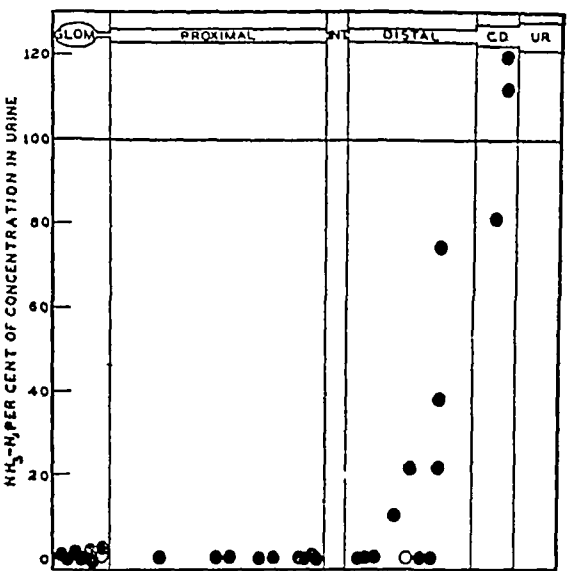


FIG. 9. Ammonia concentration of the tubular fluid at various levels in the amphibian nephron (11).

Slyke's laboratory showed in the acidotic dog that some two thirds of the urinary ammonia is derived from the amide nitrogen of glutamine, and that none is derived from urea.

Krebs and others have suggested that amino acids may be precursors of urinary ammonia, a view considerably strengthened by the demonstration of three enzymes in the kidney capable of oxidatively deaminizing selected amino acids. Dr. Lotspeich and I (12), in a series of experiments on acidotic dogs, infused the eight representative amino acids shown in table 4 in such amounts as to raise the plasma level of amino nitrogen from a normal value of 4 milligrams per cent to 20. It was found that the rate of excretion of ammonia was increased from 100 to 300 per cent by the infusion of glycine, l-leucine, d,l-alanine, casein hydrolysate and d,l-aspartic acid. Each of these amino acids is oxidatively deaminized by kidney tissue *in vitro*, by one or another of the renal enzymes noted on the right of this table. The natural isomers of

glutamic acid, lysine, and arginine have no effect on the excretion of ammonia by the acidotic dog. Neither are they deaminized by any renal enzyme. We may reasonably infer, therefore, that one function of glycine oxidase and d and l-amino acid oxidase in the kidney is the production of urinary ammonia from certain of the circulating amino acids.

What are the factors which determine the activity of this secretory mechanism, and how do they compensate for variations in the acid load on the body? From the work of Archibald it is known that the plasma glutamine concentration is the same in the normal animal excreting little am-

TABLE 4. CORRELATION BETWEEN *in vivo* EFFECTS ON AMMONIA EXCRETION OF AMINO ACID INJECTION AND THE CAPACITY OF KIDNEY BREI TO DEAMINATE AMINO ACIDS *in vitro*.

| Amino Acid         | Effect on Ammonia Excretion <i>in vivo</i> (Lotsp. and I, 12) | Deaminated by Kidney <i>in vitro</i> (Dr. Slyke, 13) | Active Renal Enzyme    |
|--------------------|---|--|------------------------|
| Glycine            | increases   | +  | Glycine oxidase        |
| l-leucine          | increases   | +  | l-amino acid oxidase   |
| d,l-alanine        | increases   | +  | d,l-amino acid oxidase |
| casein hydrolysate | increases   | +  | l-amino acid oxidase   |
| d,l-aspartic acid  | increases   | +  | d-amino acid oxidase   |
| l-isoleucine       | no effect   | -  | none                   |
| l-tyrosine         | no effect   | -  | none                   |
| l-phenylalanine    | no effect   | -  | none                   |

Actually the kidney contains a specific l-glutamate dehydrogenase capable of oxidatively deaminizing l-glutamic acid. Glutamic acid therefore constitutes an exception rather than a confirmation of the rule that those amino acids subject to oxidative deamination *in vitro* increase ammonia excretion *in vivo*. This enzyme, however, is a typical co-enzyme specific dehydrogenase and is incapable of utilizing molecular oxygen as its hydrogen acceptor, in contrast to the d and l-amino acid oxidases which are relatively non-specific and which utilize molecular oxygen directly.

monia and in the animal in acidosis excreting large amounts. Our own results indicate that there is no significant difference in plasma amino acid level in the two states. Plasma concentration of precursor is, therefore, not a significant factor.

We believe that there are no less than two significant factors which determine the rate of excretion of ammonia: first, a fairly clear-cut factor of urine pH; second, a vaguely defined element of cellular secretory capacity, which at the moment we are unable to characterize in any exact terms. The first of these factors accounts for rapid variations in ammonia output, the second, for delayed adaptational variations. The operation of both factors is illustrated in figure 10.

All data presented in this graph were obtained in experiments on one dog. Urine reaction was varied acutely over a range of pH 5.0 to 8.0 by the intravenous infusion of bicarbonate. Two groups

of data are presented those obtained from the animal in a state of normal acid base balance, and those obtained from the animal initially in acidosis, in which the ammonia secretory mechanism

this ill-defined factor of varied cellular secretory capacity

In relating the secretion of ammonia to urine pH our concept approaches that of Briggs (13)

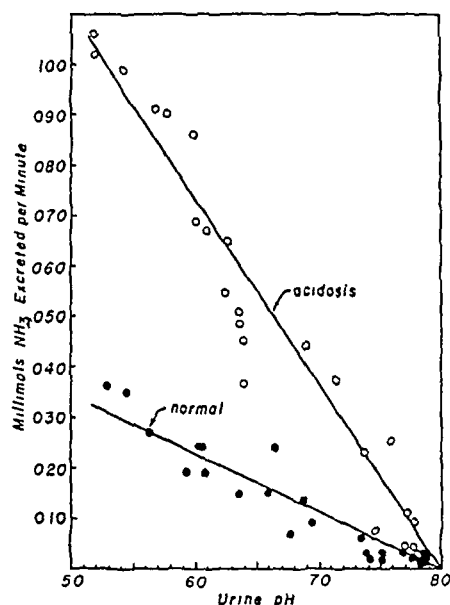


FIG 10 Relationship between the rate of excretion of ammonia and urine reaction in a normal dog and in a dog rendered acidotic for 48 hours (1)

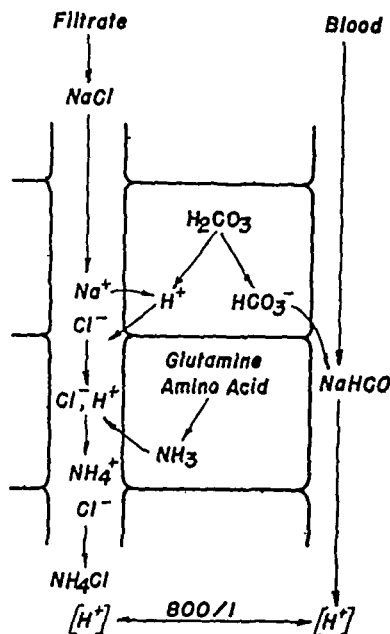


FIG 11 Nature of the cellular mechanism for secretion of ammonia (1)

#### DISTAL TUBULAR EXCHANGE MECHANISMS INVOLVED IN

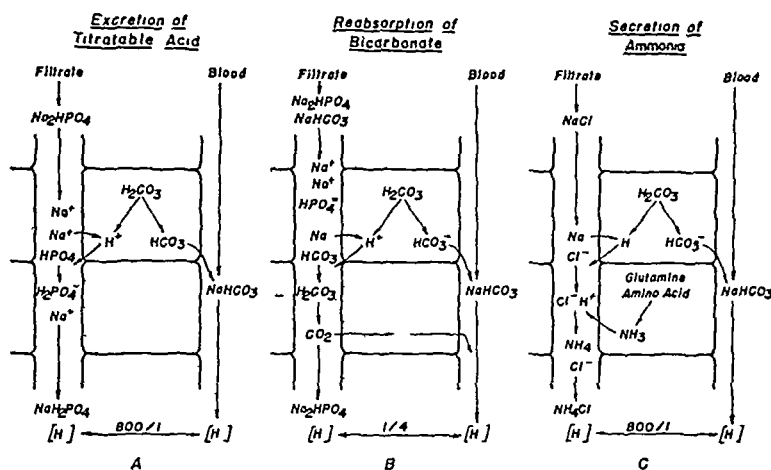


FIG 12 Diagrammatic representation of the nature of the ionic exchange mechanisms for excretion of acid and ammonia (1)

had been stimulated for a period of 48 hours. In each group there is an obvious correlation between rate of ammonia secretion and urine reaction, although the correlations differ because of

Our interpretation of the biological significance of ammonia secretion, however, differs from that of Briggs. He claims that the sole purpose of the mechanism is to protect the kidney and lower

urinary tract by partially neutralizing the highly acid urine, thus that ammonia does not contribute to acid-base regulation. Our views, which differ from those of Biggs, are explained in figure 11. If the tubular urine contained only salts of strong acids such as sodium chloride, the exchange of hydrogen ions for sodium ions could occur to a very limited extent, for the hydrochloric acid formed is highly dissociated. The high hydrogen ion gradient attained would block further transfer. The secretion of ammonia into the urine buffers this acid by binding the hydrogen ions as ammonium ions, thereby permitting the continued exchange of hydrogen ions for base. Thus the quantity of base exchanged is exactly determined by the quantity of hydrogen ions removed from the site, either as undissociated buffer acid or as ammonium ion. Fundamentally ammonia is exchanged for base mol for mol, although the exchange is indirect.

The factor of urine pH might well play its role by determining the rate of diffusion of free ammonia from the site of high concentration in the tubular cell to that of low concentration in the urine, where it exists not as free ammonia, but as ammonium ion.

The fact that ammonia production lags behind acid load, yet gradually increases more or less in proportion to accumulated base deficit, has been repeatedly observed. We are intrigued with two possible explanations: first, that the slow increase in ammonia excretion may result from a gradual compensatory increase in the concentration of glutaminase and amino acid oxidase within the tubular cells, second, that it may result from a compensatory stimulation of the existing tubular mechanism by adrenal cortical hormone. The latter view is reasonable in that acidosis con-

stitutes a threat to the base reserves of the body, and might be expected to stimulate the adrenal glands. Although work on this aspect of the ammonia secretory mechanism is in progress, no statement may yet be made as to which, or indeed whether either explanation is correct.

In conclusion I should like to point out that acid excretion, whether in combination with ammonia or as titratable acid, depends fundamentally upon the exchange of hydrogen ions formed within the cells of the distal tubule for ions of fixed base in the tubular urine. The cells can establish a hydrogen ion gradient of at most 800 to 1. If as shown in diagram A of figure 12, the buffer content of the urine is high, large quantities of titratable acid may be formed before the limiting gradient is attained. If as shown in diagram C, the buffer content of the urine is low, little free titratable acid can be formed. In consequence of the high hydrogen ion concentration developed, ammonia diffuses into the tubular urine. In essence, lacking sufficient buffer in the glomerular filtrate, the kidney forms its own buffer, ammonia. As shown in diagram B, when excess base is present in the body, bicarbonate is delivered into the distal segment in appreciable quantities. The exchange of hydrogen ions for base bound by bicarbonate reduces the formation of titratable acid. Alkaline urine is formed, containing neither titratable acid nor ammonia in appreciable quantities.

The author expresses his appreciation for the contributions of his associates, Drs. R. S. Alexander, W. D. Lotspeich, W. A. Schiess, J. L. Ayer, O. W. Sartorius, and J. C. Roemmelt, to the work described above, and to Martha Barrett, Ilse Langer, Phyllis Miner and Dorothy Calhoun for their able technical assistance, and to the United States Public Health Service and the John and Mary P. Markle Foundation for financial support.

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# American Institute of Nutrition

## ADDRESS\*

*Atlantic City, March 16, 1948*

### OPPORTUNITIES FOR WORLD BETTERMENT THROUGH HEALTH AND NUTRITION

FRANK G. BOUDREAU

*Executive Director, Milbank Memorial Fund, and Chairman, Food and Nutrition Board, National Research Council*

During the last two years the mood of the world has changed. Only yesterday millions rejoiced at the downfall of tyranny in Asia and Europe and set their faces toward the new day. Today men, women and children are perishing at the hands of their fellows in India, China, Indonesia, Greece and Palestine, and the lights are going out in Eastern Europe. Unless help comes from the outside world sixty million children will suffer from hunger and disease, crippled lives or premature death will be the fate of many during the coming years. World food supplies are insufficient to prevent hunger in many countries, even if they were evenly distributed. Political, economic and social problems threaten the breakdown of governments in some of the advanced countries. Fear dominates the world's thinking and the action of governments, which have come to resemble army staffs planning to strike first against possible attacks from former friends and enemies alike. This psychology and the acts and plans to which it gives birth can have only one result. Like the bird in the fatal spell of the serpent, mankind is being lured irresistibly into a third world war.

There is little or no agreement among statesmen concerning the plans and methods most likely to bring the peace for which all mankind longs. Many cling to old solutions like the balance of power in Europe which time and again has betrayed its disciples. Others maintain that warships, planes and guns will pacify the masses who rebel against authority because they can no longer tolerate hunger, poverty, disease and the premature death of their children. Many seem to believe that treaties of peace or the changing of frontiers will quiet the men, women and children whose violence and unrest are born of the misery of unsatisfied fundamental needs. That it is pos-

sible to escape from unpleasant realities by living on an island or continent shut off from the rest of the world by armaments, tariff barriers and secret police is the hope of a dwindling minority.

The cries of those who wish us to adopt these and other supposed panaceas resound in parliaments and echo in the press. Broadcasts from Lake Success too often sound like cracked phonograph records repeating monotonously the tunes we have heard so many times before.

If I should stop at this point in my criticism of those who are attempting to find a way out of the world's troubles, you would have the right to accuse me of standing on the sidelines and sneering at better men, for they at least are trying. This charge will be true of all scientific workers if they fail to rally to the help of the politicians and statesmen who are however feebly and inefficiently struggling almost unaided for world peace and freedom. We know that political battalions alone are powerless in the modern campaign for peace. They must be supported and reinforced by forces equipped with the most modern weapons that science can furnish. Science and technology were decisive factors in World War II. They must be fully deployed in the battle for world peace.

For the modern world, the world as we know it, is the creation of science. Science is responsible for the problems as well as the benefits of modern civilization. Science is continuing to advance on an uneven front at an accelerating pace. The release of nuclear energy illustrates strikingly that each new advance creates new problems and promises new benefits. As science has created our problems so it can solve them, the airplane transports penicillin as easily as it carries bombs. Our world's only salvation lies in using all of the resources of science in the pursuit of peace, it must use the weapons capable of influencing human behavior as well as those necessary for

\*Delivered at the annual dinner of the American Institute of Nutrition

Europe Oceania and the Union of Soviet Socialist Republics also belong to this group, but it includes only three countries in South America

The medium calorie areas include most of southern Europe, three countries in Asia, a part of the Middle East, a part of Africa, and a part of South America

The low-calorie areas include most of Asia, a part of the Middle East, all of Central America, and probably parts of South America and of Africa not covered by this survey

Generally speaking, the diets in low-calorie countries were just as inferior in the important nutrients as they were in calories

The Food and Nutrition Board of the National Research Council and the Food and Agriculture Organization of the United Nations have classified calorie levels along similar lines Both agree that the emergency subsistence level for a population should be 1900 calories, actual intake Hence in prewar years half of the world's population subsisted on a calorie intake only slightly above the level deemed barely sufficient to keep people alive for a short time in an emergency

Unrest is another characteristic of the undeveloped countries Name them over and you call the roll of the troubled areas of the world, are in which trouble now exists or in which it has originated The people of these countries furnish a ready soil for the seeds of social unrest, revolution and war They have little to live for Life expectancy is short Infectious disease is rife Food is scarce and hard to come by Toil is hard and incessant In such conditions hope burns uncertainly or flickers and dies To these people almost any fate seems preferable to their own So they are conditioned to embrace any doctrine or cause which promises release from their misery This, or a philosophy of fatalism, which causes them to refuse any effort to improve their environment, is the only outlet for their despair

The deplorable conditions in many of these countries have been aggravated by the war At the same time the war brought with it whispers of freedom and hints of better times to come So these people are on the march You see signs of it in China, Indo-China, India, Burma, Indonesia, Africa and elsewhere None of these countries can cope singlehanded with the problems of its own development None has the necessary capital to invest None has the essential scientific and intellectual institutions Technological knowledge is lacking Should the world stand aside and allow these countries to engage in the struggle unaided,

disaster might easily result Think of India increasing her productive capacity, carrying out a one-sided economic development, with the result that her population like that of England and Wales tripled in a century Disaster for India and for India's neighbors would be a certainty long before the century reached its halfway mark

In view of these world conditions it is no wonder that the subject of the development of backward countries was uppermost in the minds of the men and women who framed the Constitution of the United Nations and of the several specialized agencies Preoccupation with this subject is seen in the Charter of the United Nations and in the Constitutions of the Food and Agriculture Organization of the United Nations, the United Nations Educational, Scientific and Cultural Organization, the International Labor Office, the Fund, and the Bank These agencies have not been organized long enough to have gotten well into their stride but already FAO has sent missions to several countries to study their agriculture and to make long-term plans for agricultural rehabilitation The others will no doubt soon initiate activities designed to develop the backward countries in the fields in which these agencies operate This work will provide unique opportunities for scientific workers of all kinds to cooperate in the effort for world reconstruction If the work is to succeed, the cooperation of workers in all branches of science is essential For there is grave danger that partial attempts at development will do more harm than good The one-sided development of many colonies throws a revealing light on this problem The objectives of colonial policy in the main have been the development of these areas as sources of raw material and as markets for manufactured goods from the mother country To attain these ends it has been necessary to provide stable government, some sanitation and epidemic control, better communication and more productive agriculture

The result has been population growth without substantial increase in the levels of living Mortality, low enough to permit growth, nevertheless remains high, the expectation of life at birth falling below thirty-five years even in times of relative order and prosperity

Meantime, the fundamental nature of the agrarian family life, of native customs, religious beliefs, and educational horizons has changed little The result is that the materials out of which declining fertility grew in the West are not present In short, the modern nations of the West have

imposed on the world's non-industrial peoples that part of their culture which reduces mortality sufficiently to permit growth, while withholding, or at least failing to foster those changes in the social setting out of which the reduction of fertility eventually developed in the West. The result is large and congested populations living little above the level of subsistence.<sup>4</sup>

Population growth in these undeveloped countries may be brought under control in three ways: "Prolonged periods of political or economic chaos might result in considerable depopulation."<sup>4</sup> Conditions now obtaining in Kashmir and nearby add emphasis to this conclusion.

On the other hand, a period of peace and order in which there was a rapid advance would bring rapid and sustained growth. Such an epoch of growth could be terminated in two ways. If the essentials of the existing agrarian society are maintained, there is every prospect that growth will continue until the potentialities for increased production are exhausted. Then it will be checked by repeated catastrophes and generally increased mortality. In this case, however, large and poverty-stricken populations would be left with the potentiality for a new cycle of growth any time circumstances permitted.

If, on the other hand, a period of peace, order, and rapidly rising production were to be accompanied by a thorough and balanced modernization, we would expect the same or even faster immediate growth but a different termination. If such developments brought urbanization, industrialization, rising levels of living, popular education, and popular participation in political life, the same forces that eventually induced a declining fertility in the West would probably come into play. The population would then undergo transitional growth, perhaps tripling in the process. If events marched swiftly and studied efforts were made to induce declining fertility, perhaps only a doubling of present population would be involved.<sup>4</sup>

The prospect of congested populations like those of India and Java doubling or tripling staggers the imagination. But perhaps advance in the social sciences will reveal a way out of our difficulties. It is significant that the birth rate in Soviet Russia is said to have declined by more than thirty per cent in the ten years immediately preceding the reversal of her population policy before the second World War.

One-sided or partial development is plainly not the way to deal with undeveloped countries. We may obtain by this means a little temporary relief, but the last state of the world and of the victims in the undeveloped countries will be worse than the first. Moreover, no progress in raising levels of living will have been achieved. It will be necessary to begin once more at the beginning, all previous effort having been wasted.

The problems of these undeveloped areas must be attacked boldly and on the widest possible front, with all of the methods and techniques available to social and natural scientists. But national development cannot be imposed from without. It must be the work of the people themselves. Outsiders can help with capital in the form of long-term loans, machine tools and technical advice, if they go much further development becomes an artificial growth which dies when outside help is withdrawn. This mistake has been made time and time again by one country seeking to help another. For experience acquired in one country is not always helpful in developing another. And there is always the suspicion of self-interest when a single country seeks to assist another, suspicion only too often founded in fact.

The task of helping the backward countries to develop their whole economies is a task for the entire family of nations, working together, only the family of nations has the necessary resources, the varied experience and the enlightened self-interest.

In the United Nations the major emphasis is on the settlement of today's political problems, very little attention is being paid to the long-term problem of building up a unified world.

A social scientist who has been working with the Population Division at Lake Success has this to say:

No one can have listened to recent political disputes, both national and international, without being impressed by the amount of unnecessary disagreement. Advocates put their cases forward in such general terms, and in language containing so many ideological overtones, as to guarantee a maximum of misunderstanding.

Specialists working together can do much to limit the range of political controversy. Politicians often disagree over matters that the specialist knows have long been settled. The social analyst has a major obligation to reduce the area of controversy by posing real issues in the light of solid knowledge. (He) is in a unique position to pose the questions of a real world made of real

<sup>4</sup>Notestein, Frank W. *Population—The Long View*. Chapter in Food for the World. Harris Foundation Lectures. Chicago: University of Chicago Press, 1945.

people with problems of health, food, housing, education, and work.

If existing tensions are to be relaxed, we must make new efforts to achieve common aims. Such common aims need not be unattainable flights of fancy. In unsettled times we tend to forget that there are common aims, basic human needs and desires,—better food, better housing, better health, better education and better working conditions. To the common man throughout the world these aims are real. He seeks, not Utopia, but a little alleviation of his lot. All too often we forget that much can be done within the framework of all ideologies to fulfil the hopes of men by solving common problems of social and economic engineering. Moreover, I believe that in such work lies the means of discovering that our cleavages are less insuperable than they now appear.<sup>5</sup>

Surely it is not beyond the capacity of mankind to work together for these basic needs. If these aims were proclaimed by the United Nations as its major long-time goal, there would be a new surge of hope among the masses throughout the world. Those who guide the destinies of the United Nations will not adopt this policy on their own initiative, for by training and experience they are conditioned to a world in which the paramount issues are those which set nation against nation. No matter how humane and farsighted, their continuance in power depends upon their success in defending national points of view. Only when the people themselves insist that these real issues must be given more than lip service will this point of view prevail.

The great danger is that the present preoccupation with current emergencies will continue, and so much will be staked on the solution of each that any failure will lead men to reject the United Nations as they rejected the League of Nations. As I write, I hear over the radio or read in the press, again and again, that failure of the United Nations to enforce the partition in Palestine will lead to its downfall. How ominously like the comments heard over Corfu, or Manchuria or Ethiopia in the 1930's! Weak and inexperienced and operating in a badly divided world, the United Nations has no alternative but to face and grapple with these recurring crises. Its hands will be strengthened and its capacity enhanced by daily experience in less controversial and more fundamental fields. Observing some progress

toward better health, more adequate diets, better housing and more decent labor conditions in the undeveloped countries, and noting that the United Nations has made long-range plans for world development in terms of basic human needs, men will be less apt to despair of international cooperation when it fails, as it must on occasion, to solve a crisis which to our shortsighted eyes looms so large on the horizon of today.

There are many good reasons why those who are attempting to build a unified world should start with health and nutrition. No political settlements have meaning for hungry people, until they are fed, neither their minds nor their bodies are in a condition to contribute toward national development. When refugees from Asia Minor were being settled in Macedonia, progress was stopped by malaria which came on just when it was time to harvest the crops. Since outsiders can only help the people concerned to help themselves, it is obvious that malnourished peoples cannot succeed. This is plain to every student of nutrition who knows that men who voluntarily undergo semi starvation soon arrive at a stage when their entire preoccupation is of food, or that women who are deprived of thiamine rebel at tasks for which they volunteered. Two of the most cogent arguments for beginning a program of world recovery and development with health and nutrition are these:

Men from different countries desperately need practice in working together. Better health and nutrition are real needs in a real world. If our neighbors have better health and nutrition, nothing will be taken away from us. In fact we shall benefit, for there will be less danger of the spread of disease through our frontiers, and better nourished neighbors will become more prosperous and demand more of the goods we have to sell.

Keeping in mind the fact that the work of developing backward countries must be carried on largely by the people of these countries, anything that gives them confidence in their ability to do so and practice in the art of self help is to be fostered. Sociologists agree that health admirably fulfils this need. If the rural Chinese can be taught to dig deeper and better protected wells or to chlorinate their small water supplies, or to oil nearby ponds and destroy mosquito larvae, they will find that by their own efforts they have avoided the epidemics of typhoid fever or malaria which each season take a toll of lives in their villages. They may be led step by step to resolve

<sup>5</sup> Notestein, Frank W. International Population Problems. United Nations *Weekly Bulletin*, September 30, 1947.

more complicated issues. Eventually they may come to understand that too frequent childbirth, with its toll of maternal and infant death, is just as susceptible to their control as the polluted well or the stagnant pond.

It is easy to assert that this is the way to start the world on the road to recovery and peace but it is harder to start the ball rolling in the right direction. This being a world in which public opinion ultimately rules, the public must be enlightened on the importance of food, health, housing, good working conditions and a decent level of living for all. They must be told about conditions in the backward countries and what those conditions mean in world turmoil and unrest. They must be shown what modern science can do to curb malnutrition and disease, and told how this must be followed up by social and economic engineering to bring about a rounded development. Who is to undertake this work? Surely it is the workers in all fields of science who alone have the necessary knowledge and experience.

How should they go about it? A first step might be a meeting of one or two leaders from each of the national associations in the various fields of science to discuss and agree on ways and means of uniting all workers who use the methods of science. Membership in the American Association for the Advancement of Science is open to all persons engaged in scientific work whether in the fields of the natural or the social sciences, so this Association might sponsor the first meeting. The aim would be the establishment of a single scientific organization which would be guided and controlled by scientific workers from every field, one which would become the voice of science in America. It should be a multi-discipline organization throughout, in all its meetings and deliberations workers from the different fields of science would participate, each contributing his special knowledge and point of view. This organization would not supersede any of the specialized professional associations, it would give them all a better opportunity to make their individual contributions to the welfare of society. In view of the critical times in which we live, the organization should, in my opinion, concentrate on the contributions which science can make to the definition and solution of the problems which threaten to bring on a third World War. Science has a contribution to make to every problem real or imagined which confronts the United Nations,

but its aim should be to point out fundamental world issues and needs and to indicate what science has to offer in their solution. I am sure that agreement would soon be reached on the need to develop the backward areas of the world by every means known to science.

The establishment of such a multi-discipline organization of scientific workers in this country would soon find imitators abroad, our national organization would no doubt send missions to the different countries to encourage and assist the movement. Ultimately a World Association for the Advancement of Human Welfare Through Science would result, and this would inform the public and become the consultant of UNESCO, WHO, FAO, the Economic and Social Council and of the United Nations itself.

I do not believe that science separates men. Those who use the methods of science in their work, whether it be geology or psychology or chemistry or astronomy, have common interests and common aspirations. If they associate only with workers in their own special fields, their influence in the conduct of national and international affairs will be small. Scientific workers must not only seek to advance our powers over nature, but must also see to it that the new powers are used for the benefit of mankind. Both of these responsibilities can be carried more easily if scientific workers unite their efforts for a common purpose.

Some of you may be familiar with what is being done to set up a World Federation for Mental Health. This effort is being sponsored by three international voluntary agencies. Instead of holding an international congress at which many papers of interest to the psychologist or psychiatrist are presented, the theme "Mental Health and World Citizenship" has been selected, and studies of the different aspects of this theme are being carried out by a large number of groups in their own neighborhoods. These are multi-discipline groups, consisting of representatives of three or more professions or disciplines: psychiatrists, psychologists, general practitioners, pediatricians, nursery school teachers, social workers and public health nurses. The argument for multi-discipline groups is cogent.

Experience has shown that often when professional groups with similar interests but different backgrounds come together to discuss the same problem, the views of each are broadened and each is able to approach the problem more con-

structively. It is anticipated that pooling the experiences of the several disciplines concerned with mental health will provide a broad and stimulating program and an excellent base for future planning<sup>6</sup>

Hundreds of these commissions are at work in more than a score of countries. Each of them is concerned with the problem "What do we do to children that leads them as adults to make war, and what can we do about it?" This is a striking example of how scientific workers should proceed to deal with the problems of world recovery and peace. And it suggests one angle which I have not mentioned—that while joining together to work for the all-round development of backward areas in foreign countries, we must not forget our own. It is no part of my thesis to suggest that the advanced countries should stand idle while the backward countries catch up with them. It is their privilege to blaze the trail and to set the example of progress. Our own civilization is far from perfect, and we suffer in health, in morals and in pocket because of our backward areas with

their high fertility and mortality, inferior educational opportunity and undeveloped resources. The TVA is showing what can be done by a balanced program of development, it is not surprising that no action of our government in recent years has aroused more curiosity and more interest throughout the world.

Let me emphasize once again that we who are described as scientists or workers in the field of science, must join with all our associates and not merely with those who work in the natural sciences to make our influence, our methods and our points of view strong and effective forces in the solution of the world's most pressing problems. A physician who wrote on *Remobilization for Enduring Peace and Social Progress* has issued this challenge<sup>6</sup>

I say to you with the utmost seriousness of which I am capable that this is no time to excuse yourself from paying the debt you and yours owe the social order with some facile verbalism like 'Nothing will come of it, it can't be done.' Begin, and let it be said of you, if there is any more history, that you labored nobly in the measure of man in the XXth century of the Scientific Western World.

<sup>6</sup> International Congress on Mental Health. U. S. Bulletin 1. United States Participation in the International Congress on Mental Health.

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## AMERICAN PHYSIOLOGICAL SOCIETY

### Symposium on Physiology of Neuro-Muscular Junctions<sup>1</sup>

JOHN H. WELSH, *Chairman*

#### PHYSIOLOGY OF NEURO-MUSCULAR JUNCTIONS

JOHN H. WELSH

*From the Biological Laboratories, Harvard University, Cambridge, Massachusetts*

Since the contributions to this symposium are concerned mainly with the problem of junctional transmission between nerve and muscle in the vertebrates, it may be useful, by way of introduction, to sketch briefly the evolution of mechanisms of communication between cells. We often gain a better understanding of the biology of the vertebrates if we have some notion of the ways in which more primitive animals have achieved essentially the same ends.

Most of the protozoa have no problem of inter-cellular communication since they consist largely of single cells. They do, however, have the problem of intra-cellular coordination of their many activities and have obviously achieved a satisfactory solution to this problem. For example, the fibrillar system for the control of ciliary beat in ciliate protozoa is an effective means of coordinating the movements of these numerous organelles.

With the advent of multicellular animals the need arose for coordination of the activities of their many cells and organs. No longer could messages be conducted through continuous protoplasm and over relatively short distances, but cell membranes and long distances imposed new problems. One solution to the problems of distance and membrane barriers was to have certain cells develop elongated processes. This is seen for the first time in the coelenterates where sensory cells are found with long processes which end in close association with effector cells, or carry messages to a primitive network of neurons which is designed to conduct in all directions to muscle cells throughout the coelenterate body.

It is well to recognize that this primitive nervous system arose from cells already in existence and probably retained metabolic systems for conduction and processes of transmission, from cell to cell, that had evolved earlier.

With the origin of bilateral symmetry in the flatworms, the earlier diffuse network began to condense and the movement of animals in a given direction resulted in the beginnings of cephalization and the alignment of cell processes into nerves. From this point on throughout the animal kingdom the basic unit plan of the reflex arc is found, but the gross anatomy of the nervous system varies with the body plan of each main animal group.

Along with the development of attenuated protoplasmic pathways as direct lines of communication another type of coordinating system evolved—the endocrine system. In this system chemical substances produced by cells in one part of the organism travel by way of circulating fluids to all parts of the body. This is a kind of ‘broadcasting’ system in contrast to the nervous ‘telephone exchange’. Only cells which are properly ‘tuned in’ respond to a particular hormone. The early beginnings of the endocrine system are poorly understood, but in the arthropods such as Crustacea and insects it is found in an advanced state. The remarkable sinus glands of the crustacean eyestalks are not unlike the vertebrate pituitary in the diversity of function over which they exert control.

It is obvious in the invertebrates that a sharp distinction cannot be drawn between the modes of action of the nervous and endocrine systems, at least as regards events occurring between

<sup>1</sup> Atlantic City, N. J., March 1948

cells. Neurons are designed for rapid protoplasmic conduction, the exact nature of which is still poorly understood, but at the same time they may act on an adjacent neuron or on an effector cell through the release of chemical substances. This transmission process, since it is over short distances and is fast acting, may utilize relatively unstable molecules compared with those familiar as hormones, but chemical events at cell membranes are undoubtedly common to both endocrine and nervous coordination. It has been known, since the work of Gaskell in 1914, that chromaffin cells in the ventral ganglia of the leech give rise to nerve fibers which have an excitatory action on the pulsating lateral blood vessels and that the effects of their stimulation may be reproduced by applied adrenalin. Gaskell came close to demonstrating cholinergic neurons, since he also showed that the action of inhibitory nerves on the same vessels in the leech could be mimicked by applied muscarine.

Of the chemical substances, produced by the nervous system in invertebrates and apparently involved in junctional transmission, acetylcholine is the best known. This ubiquitous substance and the enzyme systems for its synthesis and destruction were in existence long before the first differentiated nerve cell. For example, certain bacteria are effective synthesizers of acetylcholine and acetylcholine appears to be present in protozoa. The flatworms are the most primitive animals in which large and easily demonstrable amounts of acetylcholine and cholinesterase occur in nervous tissue. Acetylcholine has been found in all groups above the flatworms where sufficient isolated nervous tissue can be obtained for assay, but that we still have much to learn concerning its exact role or roles is apparent to all who follow the work in this particular field. It should be mentioned in passing that neurons in invertebrates undoubtedly produce and release neurohumors other than acetylcholine and adrenalin. However, the work on most neurosecretory cells has

not progressed to the point of identifying the chemical nature of their secretory products.

I have tried to suggest that there are certain similarities between the nervous and endocrine systems in invertebrates as regards events occurring between cells. I have pointed out that cell boundaries presented certain problems in the course of evolution of the metazoa and that the need for fast, private communication simultaneously to many cells was met by certain cells becoming greatly elongated. Now by way of a more direct introduction to the subject of this symposium, a few words may be said about junctional transmission in the invertebrates. If there is difficulty in correctly evaluating the importance of chemical and electrical events at synapses and neuroeffector junctions in the vertebrates, this difficulty is greater in the invertebrates because of their variety and the infrequency with which they are carefully studied. If one follows the common practice of speaking of electrical or chemical transmission without attempting to account for the precise mode of action, or without consideration for the interdependence of electrical and chemical events in nerve and muscle, one may say that at some junctions, such as nerve to body muscle in leeches, transmission seems to be primarily a chemical event, while in arthropods it appears to be electrical. In the case of the control of heart beat in decapod Crustacea the evidence points to the involvement of acetylcholine in the action of the excitatory nerves on the heart while the inhibitory nerves appear to act without this kind of mediation. The question that now arises is—are there two strictly independent modes of junctional transmission—chemical and electrical—or are chemical and electrical events closely interwoven, with one or the other more easily demonstrated depending on the particular junction being studied or the interests and techniques of the investigator? We may hope that the papers to follow will help in answering this question.



## PHYSIOLOGY OF NEURO-MUSCULAR JUNCTIONS ELECTRICAL ASPECTS<sup>1</sup>

STLPHLN W KUFFLER

*Wilmer Institute, The Johns Hopkins Medical School, Baltimore, Maryland*

This discussion will be confined to the 'transmitter' problem, i.e. to the events that take place between the arrival of a nerve impulse at junctions and the subsequent setting up of a muscle impulse. No attempt will be made to refer to all of the pertinent literature, particularly to earlier now quite well known papers dealing with problems of transmission.

Since the discovery of the junctional potential at the end-plate (e.p.p.) about 10 years ago (11, 16) it has been evident that this represents an intermediary process between nerve and muscle impulses. The properties of the e.p.p. have since been studied in great detail in normal and curarized muscle. It was concluded that the e.p.p. is an expression of an excitatory process, the 'transmitter', whose time course and intensity could be analyzed. From single nerve-muscle fiber preparations it became clear that the end-plate potential normally sets up the muscle impulse by depolarizing the muscle membrane around the junction to a critical level. The main problem now is not how the muscle impulse is set up, but how the nerve impulse sets up the e.p.p. As a general scheme the following sequence can be presented: nerve impulse → 'transmitter' → e.p.p. → muscle impulse. 'Transmitter' should for the present mean no more than an excitatory process, some mechanism produced by nerve impulses to bring about the e.p.p.

At this point it may well be stressed how neuro-muscular transmission fits into the general picture of transmission in the nervous system. Events analogous to the e.p.p. occur at numerous cell junctions in a varied number of animals and organs. The similarity of processes may be seen from records of figure 1 at sympathetic ganglia in the cat, in the squid stellate ganglion and from potential changes in the cat or frog's spinal cord, as recorded from the ventral roots. Also the Limulus heart ganglion belongs in this class (29). All

these have one common feature, namely the 'synaptic' potentials which cause the propagated impulses. 'Synaptic potential' may be defined most conveniently as the potential set up transsynaptically by a presynaptic nerve impulse. It normally constitutes a link between two processes, having different properties from both.

The study of junctional potentials has so far been applied to crustaceans and vertebrate skeletal muscle (cat, frog) but has not been extended effectively to smooth muscle and the heart. The reason lies in the morphology of these structures which makes them at present 'inaccessible'. The local nature of junctional potential changes, which is normally swamped by the much larger propagating impulses and the shunting effect of surrounding tissue, make electrical recording techniques difficult even in isolated preparations like the sartorius of the frog. There is hope now that e.p.p.'s may eventually be recorded in human beings, particularly in some pathological conditions or after treatment with certain curare-like drugs.

The end-plate has become, in recent years, a physiological entity which at present cannot be strictly identified with the structure of the histologists, although progress in that direction is being made, particularly by Couteaux (7). Much interest is also focused on comparative studies of structures like the plates of the electric rays (14). The e.p.p., for instance, while originating in the end-plate which can be quite accurately located, involves also the surrounding muscle tissue which becomes depolarized before the muscle impulse is set up. Conversely the muscle impulse, if started by direct electrical stimulation, depolarizes the end-plate while it propagates past the junction. Effective membranes separating muscle from end-plate do not appear to exist (22). There are some distinctive properties peculiar to end-plates: specific chemical excitability to substances like nicotine, caffeine and acetylcholine (ACh), this is greatly increased by denervation. No peculiar electric excitability has, however, been noted. Also  $K^+$  does not show specific stimulating effects on junctional regions of frogs. With tetany or cal-

<sup>1</sup> The recent work of the author reported in this paper was aided by a grant from the National Foundation for Infantile Paralysis.

cium lack the end-plate becomes involved prior to other structures. In myasthenics for instance the junctions seem to be affected first. Curare acts specifically on this area. When a nerve impulse reaches the junction it can set up a response as large as the muscle impulse but different in character, this type of response cannot at present be produced elsewhere along a muscle fiber (21).

We know practically nothing about the basis of this specialization, but from experiments on ganglion cells in changing the chemical environment (4), similar properties in other synapses, as compared with axons, may be suspected.

The physiology of a different type of end-plate from the well-known one has recently also been investigated in striated frog muscle (24). It receives small diameter motor nerve fibers which set up a potential resembling the curarized e.p.p. of the known 'twitch system', or the prevalent local junctional potential of crustaceans. As in the latter the potential produces local non-propagated shortening of numerous muscles, it is absent in some.

The present discussion will be confined mainly to transmission in the twitch system of the frog and to the 'normal' one in mammals, the only one so far known. The analogy between these two seems complete.

The rôle of the e.p.p. in initiating muscle impulses is well shown during the progressive action of curarine (fig. 1B). The e.p.p. can be decreased by about two-thirds before neuromuscular block results and it appears that a critical minimal membrane change is required for successful transmission. This implies that the normal transmitter mechanism has a fair safety margin. Such action is, by the way, probably at the basis of the possible clinical use of this drug.

The 'transmitter' producing the e.p.p. does not cease to act after the muscle impulse has been set up but is still present for several milliseconds (msec). It produces the subsequent potential change which is confined to the end-plate and is the first to be affected by any agent which tends to block transmission, such as curare or fatigue. While figure 2A gives a rough estimate of the persistence of the normal e.p.p. a more detailed analysis has been made by a study of the curarized potential at the junction. This is largely based on two assumptions: a) the potential is built up while the 'transmitter' acts and its rate of rise and magnitude is an approximate indication of 'transmitter' intensity, b) when the 'transmitter'

has ceased, the potential declines, approximately exponentially. Such an analysis is independent of the nature of the excitatory process. In this way a total duration of about 5 msec, reaching a maximum in the first 2 msec at 18°, has been obtained for frog's muscle (13). A different approach, while applying the same principle and giving similar results, was used in studies on interaction of nerve excitation with antidromic muscle impulses. Figure 2D serves as illustration. A nerve impulse may reach the neuro-muscular junction while a muscle impulse sweeps past it, rendering it refractory. During that period no potential can be built up. As soon, however, as the membrane recovers, the surviving 'transmitter' produces a depolarization which may persist for several msec. Figure 2C shows a corresponding picture, but obtained by a different procedure. Potentials recorded at the end-plate, set up by nerve stimulation and direct muscle stimulation, were superimposed. The difference is due to 'transmitter' contribution and is confined to the end-plate region (for details see 22). The validity of such an approach is supported by experiments with applied currents or chemicals. The 'active' transmitter phase is easily reproduced by a rectangular current pulse, if applied instead of a nerve impulse on top of an antidromic muscle spike, it produces similar membrane changes while the current flows (fig. 2B).

#### NATURE OF THE 'TRANSMITTER'

This much debated subject will be discussed in relation to the e.p.p. only. Since the 'transmitter' is responsible for the e.p.p. it follows that any mechanism acting on it will affect the junctional potential also. In the first place it does not seem helpful to call the cycle of transmission purely 'electrical' or 'chemical'. It has, however, become accepted to call 'electrical' the propagation of a nerve impulse in which the mechanism of spread is initiated by current flow. The impulse cycle itself is obviously complex in nature. If, in the course of an impulse a substance is 'liberated' or ions leave the membrane or if, for instance, an enzyme system is 'activated' then the term 'chemical' is generally used. This concept seems to be derived largely from hormone secretion.

'Chemical' mechanism. How then does the ACh hypothesis fit into this picture? According to this thesis ACh should be responsible for the e.p.p. and should normally disappear at a similar rate. The main support for the theory is derived from the

following evidence and based on arguments as stated in part in 1942 by Eccles, Katz and Kuffler

ACh is liberated by motor nerve impulses, it has a powerful and rapid stimulating action on the end plate region of the muscle, it is rapidly hydrolyzed by an enzyme concentrated at or near the end plates. The rapidity and chemical specificity of the ACh effect suggest that ACh produces its depolarizing action by combining with specific chemical receptors on the surface. Curarine, while

prolonged. This actually happens and junctional potentials lasting for seconds may be obtained. The effects are particularly striking with repeated nerve excitation. Normally the 'transmitter', although outlasting the setting up of the muscle impulse, decays before the refractoriness has recovered. With eserine, however, it may persist long enough to set up repetitive, instead of single responses.

Some difficulties arise with curarine antagonism like the shortening by curarine of pro-

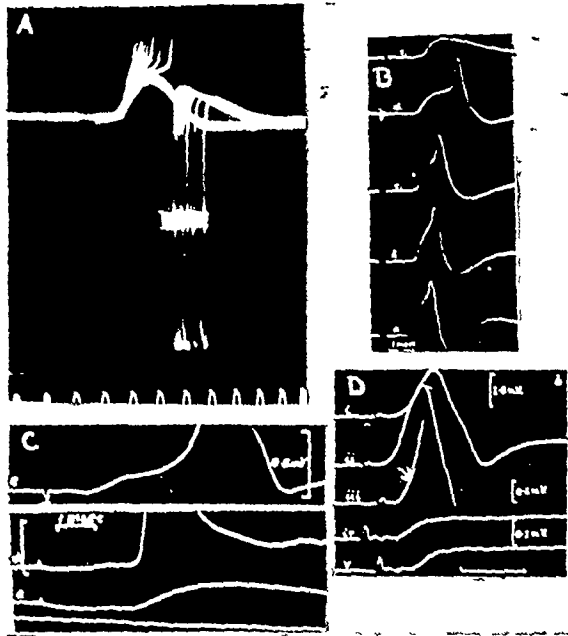


Fig. 1. POTENTIAL CHANGES AT VARIOUS JUNCTIONS. *A* Squid stellate ganglion, single synapse preparation, during development of fatigue. Nerve impulses (spikes off the record) arise progressively later from synaptic potential (6). *B* Single nerve muscle fiber preparation during progressive curarization. Muscle impulses arise progressively later from e.p.p.'s (21). *C* Cat, potentials from ventral root in response to dorsal root stimulation. *d* Spike from monosynaptic reflex discharge. *c* and *e* Spike partially and fully inhibited by preceding nerve volley. Note synaptic potential (10). *D* Cat stellate ganglion before (i, ii, iii) and after (iv, v) complete curarization. Amplification varied. Arrow in iii shows double step rise (8).

apparently not affecting the ACh liberation, opposes its depolarizing action on the muscle membrane. Since, in addition to this specific blocking action, curarine chemically resembles ACh (both are quaternary ammonium cations, as also are other substances with similar actions), it seems likely that it also acts by combining with these same chemical receptors (cf. the receptive substance of Langley).

If we assume that agents inhibiting cholinesterase (ChE) exert their action on the ACh mechanism, and not by some other property, then the 'transmitter' effect as analyzed above, should be

longed. Junctional potentials in eserimized muscle, further, for instance, even with large doses of eserine the 'transmitter' is destroyed at a fairly high rate, indicating some eserine-resistant mechanism of destruction. At crustacean junctions and in the frog's or cat's spinal cord, ACh and curarine seem ineffective although electrical events are basically similar to nerve muscle junctions, an extension of this hypothesis to such structures is at present difficult. On the whole, however, it still offers a plausible explanation for neuro-muscular transmission. It is also conceivable that ACh would be

'liberated' or 'activated' in the end-plate region, i.e. transsynaptically, by currents generated by the nerve impulse. Since, however, an antidromic muscle impulse depolarizes the end-plate region, it would follow that it also liberated a 'transmitter'. This can certainly be excluded in esterified muscle, where the typical prolonged end-plate potential change does not appear with antidromic stimulation.

*'Electrical' mechanism* What is the rôle of current flow generated by the nerve, in producing the e.p.p.? That synaptic or nerve-muscle transmis-

sion and excitability changes of identical time course. Penetration of a small fraction of the nerve impulses will suffice to cause membrane changes which may bring threshold lowering of say 90 per cent. Currents will also penetrate through a co-anesthetized region (26) and probably also through many other types of block. An essentially similar approach has been used previously by Blair and Erlanger (3), who showed that an anodal block may prevent the propagation of one nerve impulse while a subsequent one will penetrate. This observation is interpreted in the following manner.

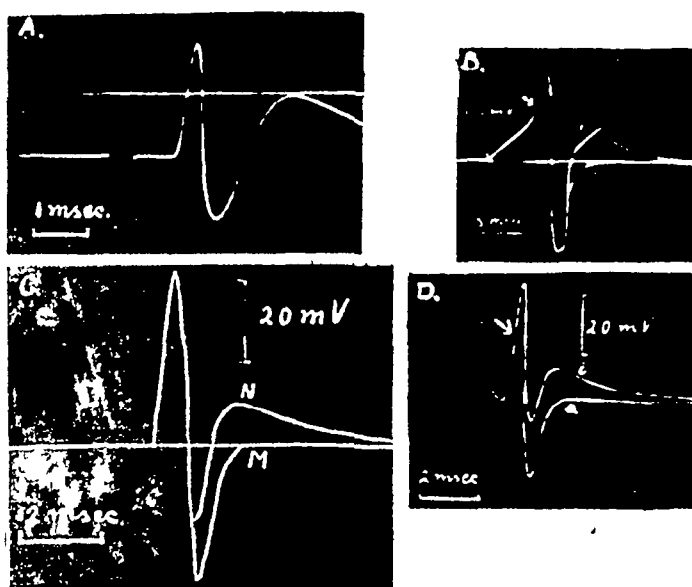


Fig 2 E.P.P. AND 'TRANSMITTER' ACTION IN SINGLE NERVE-MUSCLE FIBER PREPARATIONS. A Interface recording, accurate end plate position, shows late e.p.p. component following nerve stimulation. B Isolated muscle fiber in paraffin oil, stimulated by prolonged current pulse, two records superimposed, a, current pulse withdrawn at arrow and b, pulse continued until diphasic wave, builds up potential. Note the absence of potential addition on top of spike, compare with D below. C Preparation in paraffin, one electrode at end-plate N after nerve stimulation, M after direct 'antidromic' stimulation of muscle fiber. Records superimposed. D Same preparation and set up, a, antidromic alone, b antidromic plus nerve impulse. Action of nerve impulse starts at arrow, but adds potential only later (22).

sion is produced in such a way has been stated in one form or another for about 70 years and longer. Two experimental findings seem to be most pertinent in this connection: a) the 'electrical' nature of propagation in nerve and b) current flowing during a nerve impulse in one fiber can penetrate an adjacent fiber and can cause changes in threshold there.

The rôle of current spread in the propagation of nerve impulses has been demonstrated by Hodgkin (18). Part of the nerve impulse will penetrate through a blocked (pressure, cold) region for several mm and will cause membrane depolariza-

tion. The action currents of the first blocked impulse penetrate the blocked region, helping the following one to 'jump' across. The nature and mechanism of these phenomena is well discussed by Erlanger and Blair in a series of papers. This method was also used in the present experiments.

The analysis of Katz and Schmitt (20) and of Marazziti and Lorente de N6 (27), of the effects of penetrating currents during impulses, present impressive evidence for the efficacy of such flow from one cell to another. These and numerous other experiments on interaction of adjacent tissues during activity (1, 17, 19) greatly increase

one's confidence in the likelihood of similar events for synapses and nerve muscle junctions. A theory on these lines was recently elaborated by Eccles (9) and the principles were extended to cover also inhibition phenomena (5).

While details of Eccles' theory can be found elsewhere, a short summary of its main points follows.

1 An impulse in a pre-synaptic nerve fiber generates a current which gives a diphasic effect at the synaptic region of the post-synaptic cell with a total duration of probably not more than 1 msec in mammalian muscle and the spinal cord, initial anodal focus, with cathodal surround, more intense cathodal focus, with anodal surround.

2 This cathodal focus sets up a brief and intense local response at the synaptic region.

3 From this local response, a catelectrotonus spreads decrementally over the post-synaptic cell membrane.

4 A propagated impulse is set up in the post-synaptic cell, if this catelectrotonus is above a critical value. If it is below, then, as the local response subsides, the catelectrotonic surround decays passively.

One of the new contributions of Eccles in this scheme was the insertion of the local response, thereby obtaining a mechanism for setting up the large post-synaptic response. This would enable relatively weak currents to effect transmission.

In an attempt to test the stimulating efficiency of currents penetrating from the nerve terminals to the end-plate, a few preliminary experiments have recently been completed. They were largely prompted by Eccles' new challenging theory and by numerous exchanges of ideas with Dr. B. Katz of University College, London. a) Nerve impulses were blocked by an anode within a mm of the nerve terminals while observing the potential changes at the end-plates. b) Subthreshold cathodal shocks of different strength and duration were applied to the terminals while recording nearby at the junction. c) Neuro-muscular delay times were measured after the nerve had been stimulated near the junctions. All experiments were done on dissected preparations, containing about 3 to 4 muscle fibers with their intact nerve supply. The semitendinosus muscle was preferred for these dissections. Under the microscope nerve branches innervating adjoining fibers within a small area could easily be located. It was essential to clean the nerve fibers right down to the junctions. In single nerve muscle fiber preparations this is more difficult than in such small bundles

and furthermore the nerve can be handled in the latter more conveniently without injury, particularly by pulling. It was thus easy to place stimulating electrodes (100  $\mu$  diameter) at the nerve entry. The next essential feature is accurate recording from the adjoining end-plates. In single nerve muscle fiber preparations the initial deflection resulting after nerve excitation may be entirely composed of the e.p.p. component (21). Such a recording position then can be regarded as covering the entire end-plate region. In the present experiments only those preparations were used where an initial e.p.p. component of about 50 per cent of spike height was obtained and where a shift of 100 micra caused a marked fall of that component. Such end-plate localization was always within 0.5 mm of the dissected nerve entry. Scatter of end-plates is easily detected. The preparation was either completely surrounded by paraffin oil or the muscle fibers were kept at the oil-paraffin interface, with the nerve penetrating the interface into the paraffin (21). While both types of recording gave the same results, the latter method was preferred since more stable and accurate recording conditions were obtained.

*Blocking of nerve impulses.* Chlorided fine silver electrodes for blocking conduction could be placed conveniently at any portion of the nerve, entirely surrounded by paraffin oil, while the nerve trunk was stimulated above. Since the muscle fibers were generally innervated by not more than 2 to 3 nerve fibers, block of conduction usually occurred simultaneously in all. The block, however, could be kept just critical as determined by the following test. (a) When stimulating at a frequency around 3 per second occasionally a twitch of the muscle fibers would result, indicating penetration past the block. (b) A second impulse following 10 to 50 msec after the first one would regularly set up normal muscle activity, thus 'jumping' across the block. (3)

Nerve impulses blocked in such a manner did not exert an appreciable potential change on the nearby end-plate region. A change of 1 to 5 per cent of the normal initial e.p.p. component would have been easily detected. The stimulating efficiency of penetrating currents, under similar conditions, extending several mm past an anodal block, has been fully demonstrated in single nerve fibers by Blair and Erlanger.

*Subthreshold stimulation of nerve terminals.* At the cathode of an applied current pulse an extrapolar spatial and temporal spread of electrotonic

potential results. If a pulse of near-threshold strength is applied to the nerve portion within 1 mm of the end-plate, then the terminal region should become partially depolarized. This in turn will cause current flow from the non-affected parts into this area, part of it penetrating the nearby end-plate region. The experiment is essentially similar to the previous one, where the blocked impulse furnished the subthreshold excitation. Beside brief shocks, double condenser discharges of varying shape and duration were used. Such currents passing through the junctional region did not cause any depolarization exceeding several per cent of the normal e.p.p. size.

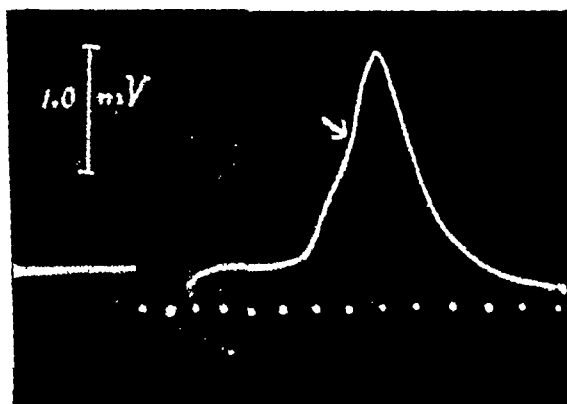


Fig. 3 SEMITENDINOSUS, ISOLATED PREPARATION of 3 to 4 muscle fibers and nerve supply. Interface recording at 22.5°C. Stimulating cathode within 0.5 mm of end-plate electrode. Stimulus 3 times threshold. Stronger stimulus does not reduce shock-response period. Arrow marks initial e.p.p. size. Time 5000 cps.

**Neuromuscular delay.** Neuromuscular delay and its analogue, synaptic delay, may be defined as the time between arrival of a nerve impulse front and the start of the e.p.p. or synaptic potential. Synaptic delays for mammals have been calculated for the central nervous system by Lorente de N6, Eccles, Lloyd and others. There seems to be agreement for values ranging between 0.5–0.9 msec. In those values no account is taken of synaptic potentials. The neuromuscular delay for frog sartorius has been calculated as between 1.1 to 1.3 msec. at 20° (13). A somewhat more accurate estimate can be obtained by stimulating the terminal nerve region while recording from the end-plates. Such a record is illustrated in figure 3. A maximal cathodal shock is followed after 1.0 msec. by the rise of the initial e.p.p. Further strengthening did not shorten the shock-response period. This time then may be assumed to consist

of conduction time, 'setting-up' time and the true delay period.

It seems reasonable to assume that the nerve fibers were excited within half a mm. of the actual end-plate. Nerve conduction time over this stretch would be negligible at speeds prevailing in the nerve trunk. It was found that conduction velocities of the motor nerve fibers of this preparation were around 30 m/sec. at 22°C. Besides the delay itself there remains then the time for 'setting up' a nerve impulse. With condenser discharges (also used in fig. 3), rising sharply to a peak in 0.05 msec. and falling to one-half in a further 0.1 msec., the earliest nerve impulses recorded near the stimulating cathode in isolated nerves arose in 0.1 msec. after onset of the condenser discharge. With shocks nearer threshold the latent period increased. Under these conditions, unless the nature of electrotonic spread is radically different over the 0.5 mm. stretch before the end-plate, one may assume that the terminal region is greatly affected practically instantaneously by electrotonic spread and subsequently by the 'active' portion of the spike, probably within the first 0.2 msec. (cf. later). There remains then a period of about 0.8 msec. delay in the preparation of figure 3. In other preparations the times calculated on this basis varied between 0.5 and 1 msec. at about 22°C. During the delay no intermediary potential changes were detected at the end-plate preceding the e.p.p. This was ascertained several times by crushing the nerve terminal, causing block. The subsequent record did not differ in its course up to the initial e.p.p. deflexion and was nearly identical with the normal baseline at just subthreshold stimulus strength.

If the nerve terminal is radically depolarized during the greatest part of the delay period, current flow into that area should occur from the surround. Further, if we assume that the e.p.p. is set up by the penetrating currents from the terminals then we must account for the lag between the two processes. Eccles believes that factors like the initial nodal focus and the time constant of the transsynaptic or transjunctional region, delaying the building up of the potential could be an adequate explanation. With stimulation practically at the end-plate, however, the duration of the initial nodal current should be extremely short, probably of the order of 0.2 msec., if that is the time assumed for the active part of the nerve impulse to reach the terminals with strong stimuli, it is uncertain therefore whether relatively

long delays (cf also later) can be explained on such a basis

In order to elucidate events in terms of current distribution, it was attempted to reconstruct lines of current flow for the present situation similar to the scheme of Eccles. The origin of the cathodal effect at the junction is different if an impulse is set up at the end-plates and propagates away, or when the impulse approaches. Figure 4 illustrates the scheme of flow when the nerve impulse is set up within a mm of the junction. There would be a short anodal focus and cathodal surround created transjunctionally lasting until the active part of the impulse reaches the endings (fig 4, 1). The subsequent situation is more difficult to visualize and is certainly different from the usual one, when the impulse approaches the end-plate (9). It is drawn in two sections. While the impulse spreads to the terminals it also extends in the opposite direction, creating a stretch of about 4 to 6 mm (at 20-30 m/sec conduction velocity) of nearly uniformly depolarized nerve. The cathodal focus at the end plate would then be provided by currents generated by the advancing impulse drawn in figure 4, 2a. This cathodal contribution would diminish as the impulse travels further away from the end-plate. During the same period the site where the impulse was initiated would start to recover and a field of current distribution as seen in figure 4, 2b, would develop and provide the cathodal focus until the terminal nerve region has been largely restored, probably within 1 msec. By that time the impulse front leaving the junction would be at least 20 mm away, thus having no or negligible effect.

It remains questionable whether such a scheme provides an adequate cathodal focus. In this connection it is interesting, however, to note that the e p p shape set up with both types of excitation (nerve impulse approaching or leaving end-plate region) is not appreciably different.

*Duration of penetrating current and of 'transmitter' effect.* Experiments on the interaction of nerve impulses and antidromic muscle impulses (cf above) provide an additional difficulty for an 'electrical' scheme. If a nerve impulse reaches the neuromuscular junction during the rising phase of a muscle impulse, set up antidromically, then it does not produce an added potential change until the refractoriness has partially disappeared. This period may be 0.5 msec (fig 2D) but can also be nearly twice as long. After that delay the 'transmitter' proceeds to affect the membrane, building up the later portion of the e p p. (Incidentally, by

this type of experiment the effective neuromuscular delay—arrival of nerve impulse to start of rise of e p p (cf above)—may be prolonged by a further msec.) According to similar analyses then the building up process underlying the e p p may last about 5 msec in frog muscle (22). In terms of current flow this implies that the nerve impulse in the terminals produces transjunctionally penetrating currents of a similar duration instead of the generally assumed short phase of about 1 msec calculated for axons (9, 20, 27).

In view of such difficulties one may postulate (9) that normally the short penetrating current sets up a local response which has a time course

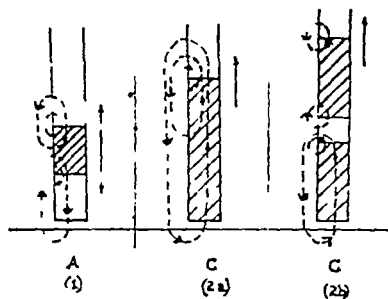


Fig 4. DIAGRAM OF CURRENT FLOW at myoneurial junction. 1. Impulse set up at nerve within 0.5 mm of end-plate. 'Active' part of impulse shown by shaded area. Brief anodal focus A and cathodal surround created at end plate. 2a and 2b. Nerve impulse has reached terminals, creating cathodal focus at C end-plates. Actual current distribution should be combination of two diagrams (see text).

similar to the subsequent muscle potential. This assumption, however, encounters difficulties in view of the interaction experiments (see above). The penetrating currents would have to set up the local response in 'completely' refractory muscle which does not permit even a small additional depolarization since the end-plate area is already depolarized by the antidromic impulse. Surviving that period the local response would 'grow' and then decay after several msec. It is questionable whether a local response can be set up or maintained under such conditions. Alternatively, the duration of the penetrating currents has to extend over the entire 'active' phase of the transmitter action.

#### GENERAL DISCUSSION

The preliminary experiments reported here in connection with 'transmitter' problems represent an attempt at elucidating the role of action currents in transmission. They are mainly based on

the assumption that penetrating currents should produce an electrically detectable membrane change. If, then, the e.p.p. is set up by current flow generated by the nerve impulse, the junctional region should be affected by certain changes in the nerve terminals provided that currents can establish an adequate circuit between endings and end-plates. In the case of subthreshold excitation of the terminal nerve regions without subsequent transjunctional potentials, it could possibly be argued for instance that the effective penetrating component was limited by the lack of 'breakdown' and consequent lowered resistance pathway. Such an argument does not hold if the nerve is maximally stimulated near the end-plate. The relatively long neuromuscular delay with the end-plate not affected in spite of such (intense) excitation nearby may be used as an argument for a discontinuity of excitatory mechanisms. However, one may also attribute to the nerve terminals very special properties preventing a quick current spread. This also would give the nodal focus more time to develop. The nerve impulse in the same fibers would travel during the usual delay period about 30 mm. If a slowing of the excitation spread in the terminal region by a factor of 30 to 50 as compared with the other parts of the axon did occur, one may attempt to account for the setting up of the normal e.p.p. by current spread. The quick tapering off of the final branches makes a greatly slowed conduction plausible. One must not assume, however, that, in order to set up a postjunctional potential, the impulse has first to reach all the actual terminal points. Since the fine nerve branches lie in apposition to the end-plate over a definite distance before terminating (7), one should expect the potential change to start as soon as the impulse enters this last stretch. A correlation between conduction velocities in nerve fibers and the delay period at junctions is of interest in this connection. It can be done accurately for nerve-muscle junctions. A sufficient number of experiments is not yet available.

The observations reported here were, in fact, undertaken in the belief that supporting evidence could be obtained for the effective transsynaptic action of current spread. They alone do not disprove that type of transmission (cf., however, below). In face of evidence demonstrating the rôle of current flow in conduction and also in producing excitability changes in adjacent nerve fibers, a similar mechanism acting across junctions but especially central synapses remains a plausible assumption. It naturally need not be assumed

that the same mode of transmission pertains to all nervous tissues. The configuration of the tissue junctions, like asymmetry of contact, also plays an evident rôle in schemes of transmission particularly in case of current spread. The absence of facilitation and the 1:1 ratio between incoming nerve impulses and transjunctional discharges in the normal 'twitch' system is in contrast to the general picture in the central nervous system. A similar mechanism of facilitation is, however, evident in the small-nerve motor system of skeletal muscle or in crustacean nerve-muscle transmission. The electrical scheme is a proposition of current density which may not be achieved in the relatively large neuromuscular junction, for instance owing to fine branching of the nerve terminals, and thus may be the reason why predictions based on observations on current spread elsewhere do not come true in myoneurial transmission. A sufficient density, however, may result at small synaptic knobs.

Other difficulties of an 'electrical' explanation have been pointed out by Eccles (9). The curare-block remains unexplained, the effect of anticholinesterases on the e.p.p. points to a rôle of an ACh-like transmitter. Long delays in some systems have to be considered (15, 30). An assumption of the duality of transmitters supplementing each other may be, to many, an obvious, but unattractive solution of difficulties.

The inhibitory nerve impulse in crustaceans reaching the end-plate region and acting on the membrane and on the linkages beyond it, i.e. by an intracellular mechanism, without changing its potential, is a further difficulty pointing out the possible ineffectiveness of mere current spread from a nerve impulse (25, 28). It emphasizes the well known fact that membranes can be greatly affected without appreciable change in resting potentials. Curarine at junctions and novocaine or cocaine in nerve or muscle being just two examples (2). There may be similar mechanisms operating in the physiological processes of the vertebrate nervous system. They are, however, rarely considered to be part of inhibitory function, i.e. nerve impulses purely inhibitory in nature, conditioning the membrane but not changing its potential. Conversely, also, sight has been lost of Gaskell's (1886) old, but several times confirmed observation, that the inhibitory action of vagus stimulation is accompanied by an increased polarization of the heart muscle. An adequate histological picture of crustaceans, showing the



end-plates receiving both types of nerve fibers, is of primary importance

*Difficulties from experiments with veratrine* A further serious difficulty of effective action current penetration into the transjunctional region arises from experiments with veratrine. These have not been discussed before in this connection. It should be an essential feature of any 'electrical' theory that the nerve impulse generates the current flow setting up the e.p.p. and, consequently, prolonged potentials in the nerve terminals should also be accompanied by prolonged transsynaptic changes. Such experimental conditions are obtained by veratrine treatment of preparations. Veratrine increases the normal after-potential in axons and certainly should do the same in the terminals. No concomitant prolongation of e.p.p.'s, however, could be observed in well controlled experiments (23). Such tests should also be applied to synapses like ganglia. This type of experiment also appears to exclude the afterpotential from a primary rôle in normal transmission. It seems significant that, in line with the present argument, veratrine does not change appreciably the short spike component of an impulse. Production of 'transmitter', therefore, should be associated with this part of the nerve impulse only, probably with the period of 'breakdown', which is unlikely to be much longer than 1 msec. To account for the analyzed 'transmitter' duration, the breakdown would have to last around 5 msec in the terminals.

It appears from the present experiments that for successful transmission the nerve terminals have to be completely depolarized, since intermediary activation does not produce any graded transjunctional changes (cf. also afterpotentials). It is conceivable that permeability changes of the nerve terminals would affect the end plate membrane lying in close apposition. Although intimate structural details are not known it may be assumed that many slowly moving ions could reach the transjunctional region even during quite a short delay period. No evidence for any intermediary tissue between terminals and muscle surface

exists (however, cf. 7). Theoretically, ions like  $K^+$  could set up the e.p.p.'s, but until good evidence for some other mechanism becomes available it seems more reasonable to assume that during the breakdown some stored ACh-like substance is released in the terminals.

#### SUMMARY

The discussion is confined to the 'transmitter' problem, i.e. to the mechanism by which the nerve impulse gives rise to the end-plate potential. Duration and intensity of the 'transmitter' is analyzed. In this connection the ACh and action current theory of transmission is briefly discussed. Some new experiments, testing the efficacy of current flow in the terminal nerve region on the transjunctional region, are presented. Potential changes were recorded from the end-plate regions of isolated preparations, while stimuli were applied within 0.5 mm to the nerve nearby. Subthreshold depolarization of the nerve terminals by applied currents or by blocked nerve impulses did not affect appreciably the end-plate membrane. Neuromuscular delay periods were measured and are discussed in relation to current spread from nerve to muscle.

It is concluded that a) action currents in the terminals are not effective in depolarizing the end-plate region. b) The 'transmitter' action occurs during the 'breakdown' in the terminals. The 'breakdown' period is too short to account for the analyzed 'transmitter' duration. Prolongation of the depolarization in the terminals does not prolong transjunctional potentials. It is thought that ions liberated during the 'breakdown' in the nerve terminals could best account for the observed phenomena.

#### ADDENDUM

Since this symposium was held a communication from Dr. Eccles has been received, stating that the electrical hypothesis cannot be reconciled with more recent experimental results on neuromuscular transmission. Eccles and his coworkers now believe that their evidence favors ACh as the sole mechanism (*Ann. Rev. Physiol.*, 1948).

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# PHYSIOLOGY OF NEURO-MUSCULAR JUNCTIONS CHEMICAL ASPECTS

GEORGE H. ACHESON

*From the Harvard Medical School, Boston, Massachusetts*

Since the beginning of the war, the chemical aspects of neuromuscular transmission have advanced at a slow pace. Little that can be recognized as fundamental has been discovered. A number of subjects connected with this field have undergone considerable development, but for the most part this development is in directions which do not contribute to an understanding of the neuromuscular synapse itself. The most significant advances in neuromuscular transmission have been in the electrical realm. The new electrical information leads us to a new perspective, enabling us to re-evaluate some of the older observations, and by a combination of old and new to push forward the frontier.

## MICROSCOPIC ANATOMY

Since the new physiological data have brought into new perspective the minute structure involved in transmission, it is particularly important now to have a clear notion of the morphology of the neuromuscular synapse. The microscopic anatomy of this region has advanced significantly.

For 90 years it has been known that the motor nerves to a skeletal muscle fiber end in an arborization at a special region of the muscle. Much attention has been given to the forms taken by the nerve endings. They have usually been examined after impregnation with silver or gold. The work of Couteaux (1) has been based on another histological method, the supravital use of the dye, Janus green. It focuses our attention on a structure which is separate from and outside of the terminal arborization of the nerve. Material which stains with Janus green is arranged in palisades. Rod-like masses at the limit of optical resolution appear to be lined up side by side perpendicular to the surface of the nerve terminals. From the ends of these which face the muscle, fine, thread-like projections extend into the surrounding sarcoplasm. Cross sections of end plates show that the nerve endings lie in gutters or grooves. The material which stains with Janus green lines these gutters on the muscle side.

This arrangement of the Janus green-staining material has been found in the mouse and guinea pig and in two species of lizard. In the frog, it is less well developed. Palisades are arranged along the course of one of the fine nerve endings which constitute the branches of the end bush described by Kuhne as characteristic for the frog.

These unusual features of the frog's neuromuscular synapse remind us of the importance of comparative observations in different species and different muscles, and in other organs of similar behavior. In the case of the electric organs, the evidence indicates that the single unit, the electroplate, is like a neuromuscular end-plate, without

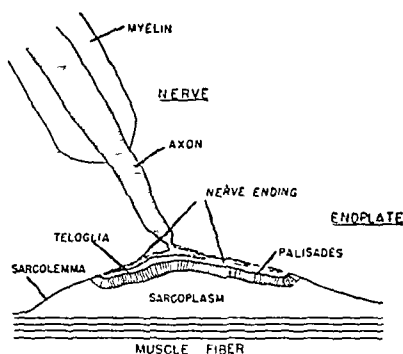


Fig. 1. DIAGRAM OF END PLATE REGION, adapted from Couteaux.

the accompanying muscle fiber, with its conductile and contractile systems. It has long been recognized that the electroplate contains palisades, lining the region where the terminal arborization of the nerve makes contact with the electroplate. The resemblance between these palisades and those described by Couteaux in the end-plate is striking. It supports the hypothesis that this material is of some physiological importance both in the electroplate and in the end plate.

The diagram shown in figure 1, adapted from Couteaux, summarizes our knowledge of the morphology of the end-plate region. Couteaux believes that a special sheath, the teloglia, with its own nuclei closely invests the nerve terminals

# CHEMICAL SPECIALIZATION OF THE END-PLATE REGION

The events of neuromuscular transmission represent physiological activity of the structures indicated in figure 1. The nerve impulse arrives above and the muscle impulse departs to either side. Some structure in this region is responsible for the end-plate potential. Is it the telodermis? Or the palisades? Or is it some structure as yet unknown?

The nerve has a physiological membrane across which a potential is maintained by oxidative metabolism. Likewise the muscle has a membrane with a membrane potential. The exterior of these two physiological membranes face each other in the region represented by figure 1. The arriving nerve impulse and the departing muscle impulse represent waves of depolarization of the respective membranes. When the sarcolemma is pierced anywhere along the muscle fiber by a micro-electrode, the potentials which are recorded indicate that the tip of the electrode is inside the place where the membrane potential is maintained. (2) Since histologically, the region lined by the palisades appears to be continuous with the sarcolemma, it seems likely that this region represents a part of the physiological membrane of the muscle.

The end-plate potential is a local depolarization of the muscle membrane. If it is great enough, it can set up a conducted disturbance in the neighboring muscle fiber. It seems reasonable to suppose that the end-plate potential occurs in the part of the membrane of the muscle immediately adjacent to the nerve endings, that is, the very region which is lined with these palisades. What, then, do these palisades contribute to the behavior of this region which makes it different from other regions of the physiological membrane of the muscle and nerve?

There is excellent evidence for a specialized function of the end-plate region. This is the part of the muscle fiber where, as Langley demonstrated (3), the stimulating action of nicotine occurs and curare prevents this stimulating action. He proposed that there exists at the end-plate a specialized material, which he designated the 'receptive substance'. In recent years it has been shown in single muscle fibers that the end-plate region is depolarized by very small concentrations of acetylcholine or nicotine or by somewhat larger amounts of caffeine (4, 5). The rest of the surface of the muscle is a thousand

or more times less sensitive to these substances. The depolarization which they produce in the end-plate region of the muscle membrane elicits conducted responses in the muscle fiber. Thus, as a result of the great sensitivity of the end-plate to these substances, they are able to stimulate the muscle fiber in very low concentrations. It seems likely that many other substances which possess a stimulating action on skeletal muscle similar to that of acetylcholine or nicotine also act by depolarizing this specialized part of the muscle membrane.

Langley demonstrated an antagonism between nicotine and curare. A similar antagonism exists between curare and acetylcholine. Curare is able to prevent the depolarization of the end-plate region by these substances and hence to prevent their stimulating action. The available evidence suggests that curare should be considered a competitive inhibitor of the reaction between acetylcholine or other substance and the receptive substance. (6) Besides the group of alkaloids collectively known as curare, a number of other substances are capable of inhibiting the action of acetylcholine and its congeners on skeletal muscle. Many quaternary ammonium cations do this. For example, the simplest of these, tetramethylammonium ion, in certain concentrations is capable of inhibiting the action of acetylcholine at skeletal muscle. But under other circumstances this substance has stimulating properties resembling those of acetylcholine. The same is true of a large number of other quaternary ammonium compounds as well as the tertiary amine, nicotine. It seems likely that all these substances act at the specialized part of the muscle which we recognize as the end-plate. And it also seems likely that they produce their actions either by depolarizing the end-plate, or by preventing its depolarization.

The actions of potassium on skeletal muscle are in some ways similar to those of acetylcholine. Potassium, too, can depolarize the end-plate. But in this instance, the ability to depolarize is not restricted to this region. Potassium depolarizes the muscle to about the same extent in all regions. Significantly, curare does not prevent the depolarization, or the consequent stimulation, produced by potassium. Magnesium makes a block superficially similar to that of curare, both at the neuromuscular synapse and in autonomic ganglia (7, 8). But magnesium differs from curare by blocking not only the stimulating effect of

acetylcholine, but also that of potassium. Calcium also has significant effects on the end-plate region, which are to some degree separable from its effects on the membranes of nerve and the rest of the muscle fiber (9, 10).

The most striking fact about the substances mentioned which specially affect the neuromuscular synapse is that all of them are cations. Of the organic compounds, only nicotine is not a strong base. It is possible that even with nicotine, the cationic form is the effective one. The end-plate region is probably merely a specialized part of the physiological membrane. The membranes of both nerve and muscle are importantly affected by cations. Sodium is not important to the maintenance of the membrane potential, but it is essential to the mechanisms underlying excitation, and conduction, and the action potential. Potassium in excess of that ordinarily found in the extracellular phase depolarizes the membrane, it is moreover, in some way pumped into the interior of these cells, presumably by a mechanism residing in the physiological or the anatomical membranes. Calcium and magnesium have significant effects on the behavior of the excitation and conduction systems of muscle and nerve which depend on the properties of the physiological membrane.

The specialization of the end-plate membrane consists in the fact that it reacts with a group of cations which do not affect the rest of the membrane of muscle and the membrane of nerve. There may, of course, be additional ways in which the end plate is specialized.

What accounts for the specialization of the end-plate in relation to cations? Two hypotheses have been proposed. It has been suggested that the end-plate lacks a protective covering which is present elsewhere. In particular it was proposed that the myelin of nerve prevents quaternary ammonium ions from reaching the essential parts of the conducting system and hence prevents an action of these substances on conduction (11). The actions of quaternary ammonium cations at the end-plate were supposed to be the same as those which might occur at the surface of the nerve if the myelin were not present. This hypothesis has no experimental support.

The other hypothesis to account for the specialization of the end plate region is that there is a chemically differentiated substance at this region which is absent elsewhere in the membranes of muscle and nerve. We can designate such a substance as the receptive substance, after Langley.

It is at present defined only in terms of its reactions in intact cells. It is therefore highly hypothetical and so it will remain until further data are obtained.

What parts of the structure of the neuromuscular synapse give it its specialization? Are the palisades the receptive substance? Or do they represent the large amount of cholinesterase which has been demonstrated to be concentrated in this region? Or finally, are they associated with some accessory physiological processes having to do with the end-plate potential?

#### THE TRANSMITTERS

What is the nature of the physiological processes of neuromuscular transmission? The new knowledge of the end-plate mechanism makes it clear that we must consider two processes: the transmission of the influence of the nerve impulse to the end-plate mechanism and the transmission of the influence of the end-plate response to the conduction system of the muscle.

At present, the second type of transmission is the easier problem to deal with. So similar is the behavior of this transmission to that of the electrical excitation of muscle fibers that the identity of the two phenomena has been generally assumed. It is proposed that the transmitter of the end-plate response is the electric current set up in the nearby muscle fiber by the local depolarization at the end-plate. The end-plate is considered to be a region specially arranged to produce this depolarization and thus to initiate muscle impulses.

The transmission from nerve to end-plate is more difficult to deal with. Recent years have brought remarkable advances in the understanding of the electrical forces involved in conduction along the nerve axon. In a number of instances, the excitatory influence of biological potentials across gaps between electrically excitable tissues has been clearly established. As a result there has been an increasing tendency to consider the transmitter of the influence of the nerve to the end-plate as the electric current produced by the arrival of the action potential at the nerve ending. These developments have tended to crowd from our minds the data gathered a few years earlier which seemed to establish that the neuromuscular transmitter was acetylcholine. It seems worthwhile to review the evidence for the two hypothetical transmitters, the electric current, on the one hand, and acetylcholine, on the other, in the

light of the present knowledge of the events of neuromuscular transmission

In the first place it is agreed that the arrival of nerve impulses at the terminal arborization is attended by the production of electric current and by the release of acetylcholine from the nerve endings

It is further agreed that acetylcholine can do to the end-plate mechanism what the arrival of nerve impulses can do. That is, acetylcholine can depolarize the end-plate and thus set up muscle impulses. It has been assumed that electric current (similar in duration to the action current of the nerve endings) can set up end-plate potentials similar to those elicited by nerve impulses. Direct evidence for this is, however, lacking. And a number of attempts to demonstrate such a fact have been negative.

In the absence of direct evidence that electric currents of the duration of action potentials of motor nerve fibers are capable of setting up end-plate potentials, we may well ask: Is the end-plate mechanism electrically excitable at all? To some it may appear unlikely that a structure which produces an electrical response should be electrically inexcitable. However, the available evidence suggests that electric organs are electrically inexcitable. Whenever electric stimuli elicit the electric discharge, the latter occurs at a delay which indicates that the stimuli were acting on the nerves rather than the electroplates. When the electric organ is chronically denervated, it becomes electrically inexcitable (12). The similarity of the electroplate to the neuromuscular end-plate is indicated by embryological, histological, physiological and biochemical data. The evidence that the electric organ is electrically inexcitable is, like that for the end-plate, negative evidence. Certainly the hypothesis that the transmitter from nerve to end-plate is electrical would be on firmer ground if it could be demonstrated that the end-plate mechanism is electrically excitable.

Are the two hypothetical transmitters produced in great enough quantity to elicit the end-plate potential? Our data are scanty. Since we do not know the electrical excitability of the end-plate mechanism, we cannot assess the adequacy of the current produced by the action potential of the nerve.

Work with single fibers has provided evidence as to how much acetylcholine is necessary to set off muscle impulses from the end-plate region. In the lizard, 5 micro-micrograms of acetylcholine

applied to the end-plate, in a droplet of about 50 times the volume of the end-plate, was the minimal effective dose (4). Since at best less than one quarter of the surface of the droplet could have been in contact with the end-plate, probably, only a fraction of the dose actually diffused through the sheaths surrounding the end-plate to the receptive substance. That this fraction represented an excessive dose is indicated by the fact that the muscle responded with a brief tetanus and the end-plate was thereafter inexcitable to nerve impulses until it had been washed. We can at present do no better than to say that a fraction of 5 micro-micrograms of acetylcholine is needed to set up muscle impulses at the end plate of a single muscle fiber.

No data are available on the quantity of acetylcholine released at single end-plates. It has been necessary to work on multifibered preparations and no direct counts of the number of nerve fibers or nerve endings involved have been made. Using data culled from the literature, I have calculated the output of acetylcholine per nerve impulse per end-plate from the gross outputs recorded by 5 groups of investigators in the literature (table 1). The quantity is  $10^{-10}$   $\mu$ g. This value is only about 1/30,000 of the minimal amount of acetylcholine necessary to stimulate muscle at the end-plate. All of the experimental errors would tend to decrease the gap. But even if the minimal dose necessary is ten times too high and the output of the nerve is ten times too low, a considerable gap remains between the two values. We are little better able to account for the effect of the nerve impulse in terms of the output of acetylcholine than we were 10 years ago when a gap of similar width was found on the basis of the minimal effective dose given by quick intra-uterine injection.

Is each of the two hypothetical mediators produced and removed quickly enough to account for the electrical events of transmission? The rapidity of appearance of the action potential is adequate, but its influence is probably too brief to account for the long synaptic delays which occur. We have no direct data on the rapidity of the release of acetylcholine. If the end-plate potential is a response to acetylcholine released by the nerve, the time course of the release must be less than the synaptic delay. With the reasonable assumption that the effect of the cholinesterase inhibitors on the end-plate potential is uniquely a protection of acetylcholine from hydrolysis, we can deduce that acetylcholine is being hydrolyzed already

when the end-plate potential is reaching its peak. The decrement of the end-plate potential indicates that if acetylcholine is the mediator, its disappearance must be almost as rapid as its release. Within these limits the acetylcholine might be present considerably longer than the action potential.

fault as indicated on the left in table 2. First, the transmitter of the influence of the nerve to the end-plate may be inadequate. In this case the end-plate potential is small and the muscle fiber fails to respond. Second, in the presence of normal delivery of transmitter, the end-plate may be unable to respond adequately. The muscle is

TABLE 1. CALCULATION OF OUTPUT OF ACETYLCHOLINE PER NERVE IMPULSE FOR EACH NERVE END.<sup>1</sup>

|  | 1<br>ACETYLCHOLINE<br>RELEASED PER<br>VOLLEY | 2<br>ESTIMATED<br>NUMBER OF<br>FIBERS<br>STIMULATED | 3<br>INNervation<br>RATIO  | 4<br>ESTIMATED NUMBER<br>OF ENDINGS          | 5<br>ACETYLCHOLINE RELEASED<br>PER NERVE ENDING PER<br>IMPULSE |
|--|--|---|----------------------------|--|--|
| Perfused organs                          |  |   |                            |  |  |
| A Cat tongue (Dale,<br>etc.)             | 10 <sup>-4</sup> γ                           | 2400 (A)  | 1:150 (D)                  | 720,000 (E)                                  | 1.4 × 10 <sup>-10</sup> γ/Ep                                   |
| B Cat ganglion (Feld-<br>berg, etc.)     | 10 <sup>-4</sup> γ                           | 3900 (B)  | 1:32 (B)                   | 1,000,000                                    | 10 <sup>-10</sup> γ/Syn  |
| Cut end of nerve trunk                   |  |   |                            |  |  |
| C Frog sciatic (Lissak,<br>Scheinfinkel) | 2 × 10 <sup>-6</sup> γ                       | 1000 (C)  |                            |  | 2 × 10 <sup>-10</sup> γ/Fiber                                  |
|  |  |   | MEAN DIAMETER<br>OF FIBERS | ESTIMATED SURFACE<br>OF NERVE IN<br>ENDPLATE |  |
| Quick-frozen nerve trunk                 |  |   |                            |  |  |
| D Frog sciatic (Muralt)                  | 10 <sup>-6</sup> γ per<br>μ length           | 1000 (C)  | 10 μ (C)                   | 500 μ <sup>2</sup>                           | 1.5 × 10 <sup>-10</sup> γ/Ep                                   |

- A Langworthy
- B Billingsley and Ranson
- C Gasser and Erlanger
- D Clark
- E Katz and Kuffler

$$\frac{\text{Quantity produced}}{\text{Quantity required}^*} = \frac{1.5 \times 10^{-10} \gamma / \text{Ep}}{5 \times 10^{-6} \gamma / \text{Ep}} = \frac{1}{30,000}$$

\* (Buchthal and Lindhard)

<sup>1</sup> In column 4, opposite 1 and B, the assumption of 2 end plates per muscle fiber and 8 synaptic endings per postganglionic cell body were made. See references 13-22.

NEUROMUSCULAR BLOCK

The adequacy of the two hypothetical transmitters of the influence of the nerve to the end-plate is illuminated by a consideration of the varieties of neuromuscular block which have been described. For a given nerve impulse arriving at the end-plate, transmission is ordinarily quantal, a muscle impulse is either set up, or not (23, 24). The events which occur during the transmission are for the most part graded. It is to the changes in these graded variables which we must look for the causes of neuromuscular block.

When neuromuscular transmission fails to occur, according to the current concept of transmission, one of three mechanisms is likely to be at

therefore not excited. And thus, despite a normal end-plate response, the muscle may fail to respond because its excitability is low or its ability to conduct is impaired. Examples of all three varieties of neuromuscular block have been described.

*Curare and other blocking agents.* When the application of a chemical substance leaves excitability and conduction in nerve and muscle unimpaired, but prevents the response of muscle to motor nerve impulses, the agent has generally been described as 'curarizing' the neuromuscular synapse, since this is what curare does. It is important to consider, therefore, whether all such 'curarization' represents the same type of neuromuscular block.

The fundamental change which accounts for

the neuromuscular block produced by curare itself is a decrease in the end-plate potential (table 2) (25-27). Transmission fails when the end-plate potential falls below a critical level. The deficiency could be due to a decrease in the amount of transmitter delivered or to a failure of the end-plate to respond to normal transmitter. If the transmitter from the nerve is electric, curare might conceivably decrease the action potential of the nerve. But curare does not affect the action potential of nerves. Or it might conceivably decrease the response of the end-plate mechanism to electric current. In the absence of evidence of electrical excitability of the end-plate, the latter mechanism cannot be tested.

TABLE 2 EFFECTS OF THREE NEUROMUSCULAR BLOCKING AGENTS ON THE FACTORS IMPORTANT IN TRANSMISSION

|                       | CURARE | POTASSIUM | ACETYLCHOLINE, ETC. |
|-----------------------|--------|-----------|---------------------|
| 1 Transmitter         |        |           |                     |
| Action current        | 0      | ?         | 0                   |
| Acetylcholine         | 0      | ?         | ?                   |
| 2 Endplate            |        |           |                     |
| Membrane potential    | 0      | —         | —                   |
| 'End-plate potential' | —      | —         | —                   |
| 3 Muscle fiber        |        |           |                     |
| Excitability          | 0 (—)  | —         | +                   |
| Conduction            | 0      | —         | 0 (—)               |

<sup>1</sup> Minus indicates that the agent above causes a decrease or impairment of the variable listed on the left. Zero indicates no effect. Question mark indicates insufficient data.

The evidence that curare does not interfere with the release of acetylcholine caused by nerve impulses has not been invalidated. If acetylcholine is the transmitter, the mode of action of curare is clearly established. Acetylcholine depolarizes the end-plate region, and curare prevents this depolarization by competitive inhibition. This is by far the simplest and most acceptable explanation of the mode of action of curare available. The facts upon which it is based represent strong support for the chemical nature of the transmitter. According to this view, curare acts on the second mechanism of neuromuscular block noted above, that is, it prevents the end-plate from responding normally. With high doses of

curare, the electric excitability of the muscle is decreased. But with doses just sufficient to block transmission, the failure of the muscle to respond does not depend on a change in electrical excitability (28, 29).

The mechanisms of various kinds of decurarization contribute to our understanding of curarization. If the curare is eliminated—that is, excreted, or inactivated, or washed out—the neuromuscular transmission is reestablished. The decurarization by azo dyes has been attributed to a special mechanism of inactivation of curare. It is proposed that curare is coupled to the diazo compound and that the coupled curare is no longer able to react with receptive substance (30).

Decurarization may be effected by catelectrotonus applied to the innervated part of a muscle (31). This decurarization might perhaps be partly due to an increase of the end-plate potential produced by the passage of current through this region. More reliance can be placed on the prediction that the curarization will be shown to depend upon a decreased threshold of the muscle fiber adjacent to the end-plate, which permits excitation of the muscle by a small end-plate potential.

The decurarization by cholinesterase inhibitors is associated with a growth in the amplitude and duration of the end-plate potential. The inadequate end-plate potential is rendered adequate, and transmission is reestablished. It is generally agreed that the effect of cholinesterase inhibitors on the end-plate potential is due to the protection from hydrolysis of the acetylcholine produced by the nerve. The phenomena do not exclude a possible excitatory role of the action potential of the nerve upon the end-plate.

When partial or complete curarization to infrequently repeated shocks is established, tetanic stimulation decurizes. Two separate phenomena are probably present here. One is the summation of end-plate potentials corresponding to succeeding nerve impulses, with the consequent reestablishment of transmission from end-plate to muscle. The other is post-tetanic decurarization (32). Since this outlasts the stimulation of the nerve for a considerable period, its dependence upon the transmitter of the nerve has been denied and less transient agents have been invoked to account for it. It has been argued that the post-tetanic phenomena, including decurarization, result from the potassium liberated during the tetanic activity of the nerve.



Injected potassium can indeed decurarize. In a recent investigation the effects of intra-arterial injections of potassium upon transmission in the curarized frog muscle were observed (33). When potassium was rapidly swept into the muscle in this way, transmission was first facilitated and then inhibited. The facilitation was accompanied by a fall of the threshold of excitation of the muscle and also by a rise in the height of the end-plate potential. An antagonism between curare and potassium has recently been demonstrated on the isolated rat diaphragm (34). Partial neuromuscular block was established by means of curare. The mechanical response to nerve stimulation was used to quantify the degree of block. The necessary concentration of curare was determined for different concentrations of potassium. The concentration of curare rose linearly with the concentration of potassium. The slopes of the curves were different at different temperatures. Extrapolation of these lines to subnormal levels of potassium concentration in the medium showed that at all the temperatures, approximately zero curare would be necessary to produce neuromuscular block in the absence of potassium. It would be most instructive to know the behavior of the membrane potential, the end-plate potential and the electrical excitability of muscle which accompany these changes. Potassium affects all of these variables.

Injected potassium releases acetylcholine in various tissues. It is not unlikely that potassium affects the transmitter, whether it be acetylcholine or action potential (table 2). The block of transmission by potassium is, like that due to curare, associated with a fall of end-plate potential. The mechanism of this change is not yet clear. But unlike curare, potassium in blocking doses decreases muscular excitability and interferes with conduction. During recovery from potassium block, the end-plate potential was observed to recover at a time when the muscle was still unable to conduct impulses (5). Thus potassium blocks by acting at least on the second and third parts of the synapse, the end-plate and the muscle.

Like potassium, acetylcholine can both stimulate and block. The stimulating depolarization of acetylcholine is, however, prevented by curare, whereas that of potassium is not. No data indicate whether or not applied acetylcholine decreases the output of acetylcholine from the nerve (table 2). Acetylcholine does not affect the action potentials of nerves. Hence, if the transmitter is

electric, the reduction of end-plate potential by acetylcholine must be due to an effect on the excitability or responsiveness of the end-plate mechanism. A striking difference exists between the blocking produced by curare and that produced by an excess of acetylcholine: in the case of curare, depolarization does not occur, in the case of excessive acetylcholine, excessive depolarization occurs. When acetylcholine is applied to a single end-plate, depolarization begins. After a brief interval, several muscle impulses are set up. Then, despite continued or increasing depolarization, the muscle no longer responds (5). This may correspond to the accommodation which occurs when catelectrotonic current is applied to the muscle. During this period of depolarization due to acetylcholine, end-plate potentials are reduced (35). With increasing concentrations of acetylcholine the end-plate potential becomes gradually smaller and block occurs. It is not clear to what extent the decrease in end-plate potential, produced by acetylcholine in these experiments, is a function of the membrane potential of the end-plate which is depressed by acetylcholine. If the transmitter is acetylcholine, prior reaction of applied acetylcholine with the receptive substance would be expected to reduce the degree of depolarization contributed by a normal quantum of acetylcholine delivered from the nerve.

Neuromuscular block with acetylcholine occurs when the end-plate potential is slightly greater than that which occurs at block by curare. It may be argued from this that the muscular excitability is slightly increased by acetylcholine. A continuing end-plate depolarization may be responsible for this change in the excitability of the nearby muscle relative to the end-plate potentials.

The failure of transmission, which occurs at low frequencies of stimulation of the nerve in the presence of cholinesterase inhibitors, is probably due to an accumulation of acetylcholine and a consequent continuation of end-plate depolarization. In frog muscle, local contracture in the end-plate region of the muscles has been observed under these circumstances (36). When contracture occurs, conduction along the muscle fiber in this region is blocked. Hence contracture in the end-plate region represents one aspect of neuromuscular block, in example of a deficiency of the third part of the system, the muscle.

One wonders how nicotine, tetramethylammonium, and all their congeners act on these variables of the neuromuscular synapse in producing their

block. Do they, like curare, block by a selective inhibitory action, preventing the mediator from depolarizing the end-plate? Or is their block associated with a more complex effect, like that of acetylcholine? The latter seems more likely. Their action is usually designated as curariform. But unless it is established that their action is identical with that of curare, this term is a misnomer. We have reached the point at which by proper analysis it can be determined how these substances produce neuromuscular block. Until this analysis is made, they should not be called curarizing agents, but, more generally, agents which produce neuromuscular block.

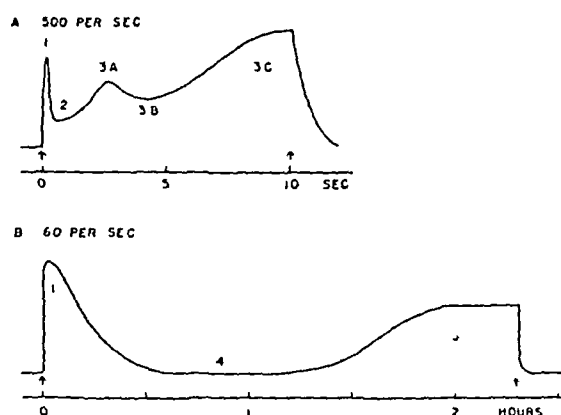


Fig 2. DIAGRAMS OF MECHANOGRAMS obtained from leg muscles of cats upon stimulation of their motor nerves at rapid frequencies.

The fundamental difference between curare and these other blocking agents seems to be their effect on the membrane potential. Unlike the other agents, curare does not depolarize, at least in a normal frog muscle, and it does not ordinarily stimulate muscle. In lizard muscle, however, a depolarization of the end-plate has been reported (4) and, in denervated mammalian muscle, di-tubocurarine is capable of stimulating (37). Denervation renders the muscle much more sensitive to acetylcholine and similar stimulating substances. Evidence from frog single fibers suggests that even in denervated muscles, the end-plate region remains specially sensitive to depolarization and stimulation by acetylcholine (5). One wonders whether, in lizard muscle, curare is capable of stimulating and whether, in denervated mammalian muscle, curare depolarizes.

*Other types of block.* Other forms of neuromuscular block are seen when the motor nerve is stimulated rapidly for appropriate periods of time. When 300 to 500 maximal stimuli per second

are applied to the motor nerve of a leg muscle in the cat, the muscle contracts vigorously but briefly. The characteristic mechanogram is indicated diagrammatically in figure 2A. The successive rises and falls of tension during continued stimulation have been designated the stages of neuromuscular transmission, by numbers, the first rise *stage 1*, the first fall *stage 2*, and the second rise-fall-rise sequence *stages 3a*, *3b*, and *3c*. In the series so named, there are two which represent failure of neuromuscular transmission, namely, *stage 2* and *stage 3b* (38). During these stages of failure the nerve is able to carry impulses unimpaired. The statistical spike height of a region of the muscle changes proportionately with the tension. Therefore the inference is made that these stages of failure depend on deficiencies at the synapse. It is possible to account for at least one of these stages of neuromuscular failure, *stage 3*. Little more than the simplest description is known about *stage 2*.

In the presence of curare *stage 3b* is diminished. In the presence of cholinesterase inhibitors, it is augmented and occurs at notably lower rates of stimulation. If acetylcholine is injected, *stage 3b* is augmented. The acetylcholine output of excised nerve is highest at first and diminishes with continued stimulation. In the superior cervical ganglion a similar failure occurs; it is affected similarly by acetylcholine, cholinesterase inhibitors, and curare (39). When the ganglion is perfused with eserinized Locke's solution, this stage of failure occurs when the acetylcholine output of the nerve is high and disappears as the acetylcholine output falls. When insufficient substrate for acetylcholine synthesis is available, this stage does not appear in the ganglion. There can be little doubt that *stage 3b* is due to excessive acetylcholine produced by the rapidly repeated nerve impulses. The mechanism of this type of neuromuscular block has already been discussed above. A close correlation of end-plate potential with transmission in these stages has not been made, but it is known that successive end-plate potentials build up in the first seconds of rapid stimulation of the motor nerve, making an increasing degree of end-plate depolarization (40).

When the motor nerve is stimulated at a slower frequency, say 60 to 120 per second, a slower series of changes is apparent (41). The mechanogram is diagrammatically represented in figure 2B. *Stage 1*, the initial rise of tension, is of course present. At this frequency of stimulation the

other early stages are absent. The tension gradually falls as a new and different stage begins, *stage 4*. The tension falls to almost zero and stays there for many minutes. Between *stage 4* and *stage 3b*, several significant contrasts are found. Transmission in *stage 3b* is improved by curare, but transmission in *stage 4* is made worse by curare. Transmission in *stage 3b* is made worse by acetylcholine and cholinesterase inhibitors, but transmission in *stage 4* is improved by these substances. As contraction becomes progressively weaker in *stage 4*, acetylcholine injected during stimulation makes progressively greater increments of contraction. In contrast to *stage 3b*, the acetylcholine content of the motor nerve is low in *stage 4* (42, 43). A similar stage of failure occurs in autonomic ganglia. As transmission across the ganglionic synapses progressively fails, the output of acetylcholine released into the perfusing solution progressively declines (44). When the perfusing solution lacks substrates for acetylcholine synthesis, *stage 4* occurs sooner, and if substrate is added, the transmission is temporarily restored. It seems certain that the failure of transmission in *stage 4*, both in the ganglion and in the neuromuscular synapse, is due to a decrease in the output of acetylcholine from the presynaptic nerve. On the other hand these frequencies of stimulation produce no appreciable fatigue in the action potential of the nerve. These facts represent strong support of the essential role of acetylcholine as the transmitter.

If stimulation is continued during *stage 4*, the tension finally rises again and may eventually reach 60 per cent of the height of *stage 1*. This reestablishment of transmission has been called *stage 5* (fig. 2). The tension may remain at this level for hours. *Stage 5* has been attributed to a gradual increase in the acetylcholine output of the motor nerve. When a nerve is rapidly stimulated for 30 minutes, it loses much of the acetylcholine it originally possessed, but in the succeeding minutes of rest, it synthesizes acetylcholine to a higher level than it originally had. The longer the nerve is stimulated, the greater is this increased rate of restorative synthesis. If nerves taken during *stage 4* and *5* are frozen without the opportunity of restoring their depleted acetylcholine, it is found that nerves contain more acetylcholine in *stage 5*, when transmission is being reestablished, than in *stage 4* when transmission is absent. The action potential of the nerve and the excitability of the muscle do not change as *stage 5* supersedes *stage 4*. The behavior

of the end-plate potential has not been examined. Thus the failure of transmission in *stage 4* depends primarily on a deficiency of the output of acetylcholine from the nerve and the restoration of transmission in *stage 5* depends on an increase in the output of acetylcholine. Low calcium and procaine are other agencies which also appear to diminish the acetylcholine output of the nerve, the neuromuscular block produced by these substances is associated with changes in the other phases of transmission as well (45).

Failure of neuromuscular transmission has been attributed to failure of production of acetylcholine by the nerve also in Wallerian degeneration (46-48). The phenomena are parallel in the neuromuscular synapse and the autonomic ganglia. When the presynaptic nerves are cut, little change occurs in the function of the axons peripheral to the cut or in transmission, within the first day. On the second and third days there occurs a progressive failure of transmission. By the end of the third day transmission is absent. Yet the action potential of the nerve remains unaffected at this time and for more than a day after transmission has failed completely. The acetylcholine content, release, and synthesis of these nerves, however, is parallel to the transmission, it is normal during the first day and falls rapidly during the next two days. The cholinesterase content of the synaptic region and the thiamine output of the nerves decline at a much slower rate. From the present evidence, this kind of neuromuscular failure seems to be due in large part to changes in the first phase of the synaptic series, the production of transmitter by the nerve. What happens to end-plate potential and muscular excitability is unknown.

#### ADEQUACY OF THE HYPOTHETICAL TRANSMITTERS

The adequacy of the two rival transmitters of the influence of the nerve on the end-plate, the action potential and acetylcholine, is summarized in table 3. A plus indicates that behavior of the respective transmitter is adequate to account for the behavior of the synaptic mechanism. A minus indicates inadequacy. A question mark indicates insufficient data on which to decide. The plus in parentheses opposite number 3 under acetylcholine refers to the facts that we know from the effect of cholinesterase inhibitors on the end-plate potential that acetylcholine is present at the peak of the end-plate potential, but we don't know that it is present earlier. The parentheses around the plus under acetylcholine

opposite number 6 refers to the fact that direct data on reduction of acetylcholine output by low calcium exist only for autonomic ganglia

Acetylcholine is sufficient to account for most of the phenomena. For some of them, for example, stage 4, and the transmission failure of Wal-

TABLE 3 ADEQUACY OF THE TWO HYPOTHETICAL TRANSMITTERS OF THE INFLUENCE OF THE NERVE TO THE LIND-PLATT<sup>1</sup>

| TESTS                           | ACTION CURRENT | ACETYLCHOLINE |
|---------------------------------|----------------|---------------|
| 1 Depolarizes endplate          | ?              | +             |
| 2 Quantity produced             | ?              | -             |
| 3 Rate of appearance            | +              | (+)           |
| 4 Rate of disappearance         | -              | +             |
| 5 Effect blocked by curare      | ?              | +             |
| 6 Quantity decreased by calcium | ?              | (+)           |
| 7 Stage 2                       | ?              | -             |
| 8 Stage 3b                      | ?              | ±             |
| 9 Stage 4                       | ?              | +             |
| 10 Stage 5                      | ?              | +             |
| 11 Wallerian degeneration       | ?              | +             |
| 12 Post-tetanic phenomenon      | -              | -             |
| +                               | 1              | 7+ (2)        |
| ?                               | 9              | 0             |
| -                               | 2              | 3             |

<sup>1</sup> + = adequate, ? = unknown, - = inadequate

lerian degeneration, one might almost say that acetylcholine is necessary to account for the phenomena. For the other tests, some other transmitter might suffice. Evidence for such transmitters at this synapse simply does not exist, but new evidence for some other transmitters

could make acetylcholine unnecessary to account for the phenomena.

An experiment reported by deCastro will illuminate this possibility (49). It involves, not skeletal muscle, but the somewhat similar superior cervical ganglion. De Castro cut the cervical sympathetic nerve and induced regrowth to the ganglion of fibers from other cut nerves. He was able to find functional innervation of the ganglion cells from a number of nerve trunks. One such nerve trunk was the vagus. If the vagus is cut central to the nodose ganglion, the efferent fibers in the peripheral trunk degenerate. The afferent fibers, whose cells are in the nodose ganglion, do not degenerate, but start growing at their cut ends. De Castro was able to establish functional connections between these regrowing afferent nerve fibers from the nodosum and the cells of the superior cervical ganglion. Now the nodosum is similar to dorsal root ganglia, and dorsal root fibers are not cholinergic; they do not contain acetylcholine (50). If those afferent fibers of the vagus which establish functional connections with the superior cervical ganglion could be clearly established as not containing or releasing acetylcholine, the transmission in this re-innervated ganglion would occur in the absence of acetylcholine as transmitter. Acetylcholine would not be necessary for the transmission. Some other transmitter would be sufficient. The data upon which such a conclusion could be made have not yet been demonstrated in the re-innervated ganglion. Certainly they have not been demonstrated in skeletal muscle. Hence we can still rely on the conclusion that acetylcholine is the transmitter. The possibilities brought up by the experiment of De Castro represent a challenge to a renewal of the controversy.

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# PHYSIOLOGY OF NEURO-MUSCULAR JUNCTIONS CLINICAL ASPECTS

A M HARVEY

*From the Johns Hopkins Hospital, Baltimore, Maryland*

The clinician as well as the physiologist has been stimulated to renewed activity in this field. Crucial experiments of a physiological nature rarely can be performed in human subjects, but the clinical investigator, aided by the recent technical advances in the field of neurophysiology, is now in a position to provide more quantitative studies in human subjects which the experimental physiologist must be able to account for within the scope of his interpretation of the mechanisms normally concerned in neuromuscular and central nervous system function. The great variety of disease processes in man provides a host of ready-made experimental situations in which nature by altering some phase of the functional process has created a test object which the ingenuity of the experimental physiologist frequently cannot duplicate. Recent advances in biochemical methods and in neurophysiological techniques have opened a broad field for investigation of diseases of the nervous system as well as the various factors in the normal human subjects which must be maintained under perfect control if the level of neuromuscular and central nervous system excitability which means normal function is to be maintained. One cannot hope in the time allowed, nor does it seem desirable, to try and review the clinical aspects of this field in a comprehensive manner. It seems more appropriate to draw on certain examples which illustrate the unique rôle which clinical investigation may play in this realm of physiology and to point out briefly certain recent advances in technique which increase the opportunities for study.

In order to analyze the events taking place when the nerve impulse excites the muscle fiber at the motor end-plate, one must have an accurate estimate both of the number of nerve fibers stimulated and of the number of muscle fibers which respond to each nerve stimulus. In animal experimental work this is done by stimulating the motor nerve with a strength of current sufficient to cause all of the nerve fibers to respond to each stimulus, but not strong enough to cause repetitive firing of the nerve fibers to a single stimulus. The muscle action potential recorded

by placing one lead in the tendon and the other in the belly of the muscle remains reasonably constant when the temperature and initial tension of the muscle are kept at a fixed level and serves as an index of the number of fibers concerned in the contraction. It has been shown possible to duplicate the conditions of animal experiments by studying the responses evoked in human muscle by slightly supermaximal stimulation of the nerve supplying that muscle (5). Muscle action potentials in a normal subject were recorded from the hypothenar eminence. In successive records the strength of the ulnar nerve stimulus was increased by 10 per cent. One record showed the development of the maximal potential and further increases in stimulus strength produced no further increase in this muscle action potential.

In order to test the validity of this method for obtaining reliable qualitative and quantitative data in the study of neuromuscular function in human subjects, the next step was to try and reproduce in detail certain well documented animal experiments. The action of curare seemed suitable for this purpose. In a study of the effects of small doses of curarine on neuromuscular conduction in the cat, Brown found when two maximal nerve volleys were set up at varying time intervals that, as the volleys became separated by a period of about 50 milliseconds, there developed an increasing diminution in the response to the second volley which reached its maximum at intervals of between 200 and 400 milliseconds. At that interval the second volley might evoke a response only one-half the size of the first. During a tetanus there was a progressive reduction in the size of the first 10 responses which showed an exponential decline to 25 per cent or less of the original potential.

Following the intravenous injection of tubocurarine, effects were observed in the human subject using this method of study which closely paralleled those observed by Brown in the partially curarized animal. The potential size decreased in proportion to the level of curarization and a single stimulus was followed by a long-

lasting depression similar in its degree and time course to that seen in the cat. When the nerve was stimulated repetitively at a rate of 10 to 15 per second, the successive spike heights showed a progressive decline to less than 50 per cent of their original value. This seemed to provide rather convincing evidence of the value of this type of recording for the study of neuromuscular phenomena in the human subject.

It has been recognized for many years that the distribution of the muscle weakness and the clinical characteristics of the disease known as myasthenia gravis resembled the changes produced in the experimental animal by the administration of curare. The additional knowledge that curarizing substances when given in small amounts to patients with myasthenia gravis greatly exaggerated the symptoms and the fact that the neuromuscular disturbance in this disease was alleviated by the administration of neostigmine, a drug which was known to antagonize the effects of curare, suggested that this resemblance of myasthenia gravis to partial curarization was more than superficial. It seemed desirable, therefore, to see whether more detailed and quantitative data could be obtained in support of this suggested resemblance.

Facilitation and depression at the neuromuscular junction have been described in animal experiments during partial block induced by small doses of curare. Bremer and Holmes working with partially curarized frogs gave a detailed description of what was called *addition latente*. Each nerve impulse arriving at the neuromuscular junction leaves behind it a process susceptible to summation so that with repeated impulses a certain liminal value is obtained which excites the contraction of more muscle fibers than are excited by a single, maximal nerve volley. Brown extended these observations and showed that this period of facilitation lasted for 50 to 60 milliseconds and was followed by a period of depression of neuromuscular conduction which lasted for more than a second. Thus, there are two possibly antagonistic processes set up by activity at the neuromuscular junction, the one tending to facilitate the passage of subsequent impulses and the other of longer duration having the opposite effect. Both of these phenomena can be demonstrated in the myasthenic patient by recording the action potentials of the muscle abductor digiti quinti evoked by supramaximal paired stimuli to the ulnar nerve (6). A patient who had myasthenia gravis had had no neostigmine

for a period of 53 hours. In the first record the second response was 19 per cent greater than the first. At the second interval it was 15 per cent greater than the first and in the third record at 70 milliseconds the second response was 99 per cent of the first. At a further interval of 160 milliseconds, the second response was much smaller than that following the first stimulation. In other words, the second of a pair of maximal stimuli evokes a greater response when it follows the first in less than 70 milliseconds, but at longer intervals a depression of the second response appears. The other point which such records demonstrate is that in certain cases of myasthenia gravis there is a partial block in neuromuscular transmission similar to that seen in partial curarization. This partial block allows one to demonstrate these phenomena of facilitation and depression which probably occur normally following activity at the neuromuscular junction.

In very severe cases the degree of neuromuscular block is greater. The phenomenon of depression following the passage of a single impulse increases in degree and masks the facilitation process. The following illustrates the results in a patient with severe myasthenia gravis in whom neostigmine had been withheld for 72 hours. When the stimuli were 64 milliseconds apart there was a very profound depression of the second muscle action potential. Neostigmine administration increased slightly the response to a maximal stimulus and decreased the depression of the second response to a pair of stimuli but it was not capable of bringing about anything like a normal state of neuromuscular function.

Eserine and its analogue neostigmine have several characteristic actions upon the neuromuscular mechanism. In eserimized muscle the tension of the twitch in response to a single maximal motor nerve stimulus is greatly enhanced. The muscle action potential under these circumstances is converted into a short asynchronous salvo indicating a repetitive response of the muscle fibers to the single stimulus. Furthermore, the excitation of eserimized muscle by a train of motor nerve volleys occurring at a frequency greater than 6 per minute results in a progressive depression of the twitch tension. Stimuli applied to the motor nerve at a rate of 50 per second cause a brief contraction followed by relaxation of the muscle. That a depression of neuromuscular conduction results from the accumulation of a paralyzing concentration of acetylcholine is suggested by the observation that the

enhanced responses of eserimized skeletal muscles to low frequency single nerve volleys are abruptly depressed by the concurrent intra-arterial injection of a minute amount of acetylcholine

It seemed of interest to study the effects of these drugs in normal human subjects and in patients with myasthenia gravis using the brachial artery as the route of administration and studying the function of the muscle abductor digiti quinti in response to stimulation of the ulnar nerve. The repetitive response described in the experimental animal can be reproduced with ease in the human subject. Here one sees the repetitive discharge evoked by a single motor nerve volley after the injection of 1 mg. of neostigmine into the brachial artery. The voltage of the initial potentials is the same before and after the drug was given. Perhaps the most striking effect following intra-arterial injection of neostigmine was demonstrated by giving pairs of supramaximal motor nerve stimuli separated by varying intervals of time. When the time interval between these paired stimuli was sufficiently brief, the muscle action potential evoked by the second stimulus was significantly lower in voltage and shorter in duration. At the earliest interval at which the two responses could be clearly differentiated the depression of the second potential was greatest, and with increasing intervals it rapidly became less, apparently being indirectly proportional to the time interval separating the two volleys. This depression following neuromuscular activity in the presence of neostigmine has an entirely different time course from that seen in the partially curarized subject or in the patient with myasthenia gravis.

Myasthenia gravis is a disease which is characterized by the reversibility of the process either spontaneously or following the administration of certain chemical substances. It may now be of interest to demonstrate briefly what changes take place when a remission is induced in this disease (7). The following illustration is from a patient with severe myasthenia gravis who had a remission following the removal of the thymus gland and shows two of the changes which occurred. The voltage of the muscle action potential in response to a single maximal motor nerve stimulus greatly increased. In addition, the ability to induce repetitive discharge following the injection of neostigmine into the brachial artery appeared. In these instances it could not be brought about before operation with doses up to 3 mg., while following operation it was easily induced with 1

mg. In another patient prior to thymectomy, without neostigmine, there was a marked depression of the transmission curves. Slight improvement was evident after the administration of neostigmine but the slope of the curve was the same. After thymectomy, before neostigmine administration, a depression of the second response was still present but was now of minimal degree. After the administration of neostigmine there was a different slope to the curve approaching that seen in the normal subject.

Di-isopropylfluorophosphate proved to be a very interesting drug for study in view of its peculiar ability to inactivate cholinesterase irreversibly. When injected in small amounts into the brachial artery of normal human subjects the effects on neuromuscular transmission, as measured by the electrical response of the muscle to maximal nerve stimuli, is essentially similar to that seen following a similar administration of neostigmine (1). In the electromyogram of a normal subject before and after the intra-arterial injection of 0.5 mg. of DFP, one sees the development of repetitiveness to a single stimulus, depression of the second response to two stimuli and the varying responses to a train of stimuli. Thirty-five minutes after the injection of DFP, 9 units of curare were injected into the same artery. The repetitive response to single stimuli disappeared completely and had not returned 30 minutes later. The voltage of the response to a single stimulus was reduced immediately following the curare, but during the period of observation slowly returned to the normal voltage. When virtually normal voltage had returned the repetitive response was still absent. This drug is likewise capable of repairing the defect in neuromuscular conduction in myasthenia as shown in the following illustration. In this experiment one was able to demonstrate by the additional injection of neostigmine a deterioration of neuromuscular conduction with the development of repetitive discharge similar to that seen in normal subjects. The train of events represented might possibly be interpreted to result from the additive anticholinesterase effects of an initial dose of DFP followed by a subsequent dose of neostigmine which permitted the development of a poisoning concentration of acetylcholine at the neuromuscular junction.

In the experiments in which DFP was injected intra-arterially it was noted that the effect was appreciably less in the patients who had received neostigmine shortly before. Also it was observed



that when DFP was given intramuscularly to the myasthenic patient, who was receiving his regular neostigmine medication, the effect of the DFP was reduced. These observations suggested that neostigmine inhibited the effect of DFP. To analyze this relationship in more detail, 2 patients with myasthenia gravis were given on five occasions an injection of DFP into the brachial artery at a time when they had received no neostigmine during the preceding 24 hours. Injections of DFP were repeated after an interval of one week in the opposite brachial artery at a moment when the patients were at the point of maximal benefit from an intramuscular injection of 2 mg of neostigmine. When the patient had had a recent injection of neostigmine the intrarterial injection of DFP did not produce the lasting increase in muscular power which followed the administration of DFP alone. These observations suggest that neostigmine and DFP may compete for a common site of action and that when neostigmine is present at this site it blocks the action of DFP.

Many attempts have been made to demonstrate a chemical component involved in synaptic transmission in the central nervous system. No crucial evidence has yet been introduced. Because of the dramatic effects of DFP on the cholinesterase activity in the central nervous system the following experiments done in normal human subjects are of some interest. (3) The daily intramuscular injection of DFP in normal subjects, who were not receiving any other medication, usually resulted in the development of symptoms referable to the central nervous system including excessive dreaming, insomnia, restlessness, increased tension, emotional lability, subjective tremulousness, nightmares, headache, increased libido, giddiness, drowsiness, paresthesias, mental confusion, visual hallucinations and pains in the legs of scatic distribution. These symptoms were not significantly affected by the administration of neostigmine, but were diminished to some degree by the administration of atropine. Electroencephalograms taken during the period in which these symptoms were present showed an increase in the potential size and in the frequency and irregularity of the rhythm. In many instances there was the appearance of abnormal waves similar to those seen in patients with grand mal epilepsy. These electroencephalographic changes were promptly reversed by atropine and were not affected by neostigmine or curie. All one can say is that in these experiments central nervous system effects have been produced by diisopropyl-

fluorophosphate which to date has been shown to have no other important action than its ability to destroy cholinesterase. The production of these central nervous system effects by a compound which has been shown to inhibit cholinesterase within the brain suggests that the acetylcholine cycle does play a positive though as yet undefined role in central neural function. The point which I wish to emphasize here is that in the human subject one is able to study the effects of this drug without the introduction of any possible conflicting factors such as anesthesia or any operative procedure and in the normal unanesthetized human subject one is able to record the subjective alterations in normal central nervous system behavior as well.

At this point I would like to review very briefly certain additional techniques by which the clinical neurophysiologist may be able to obtain more detailed knowledge of central nervous system function and to study in a more precise fashion the effects of various diseases and chemical agents on this function.

Piper in his monograph on *Electrophysiology of Human Muscle* was the first to suggest that conduction velocity in a peripheral nerve may be measured by stimulating the nerve at two different sites, measuring the latencies of the muscular responses evoked by each shock and dividing the latency difference by the distance between the points of stimulation. In recent experiments Hodes (9) has used this method of studying conduction velocity of the skeletal motor nerve fibers supplying parietic muscles. The electrical studies consisted of the percutaneous application of single supra maximal shocks to the motor nerve and the recording of the muscle action potentials thus produced, as described earlier in this discussion. Conduction velocity was measured by the method described by Piper. Hodes obtained action potentials from some 500 parietic muscles of poliomyelitis patients and expressed them in percentage of the voltage of corresponding normal muscles and compared them with the muscle strength estimated by clinical testing. Such data showed that the size of the action potential indicated the severity of the muscular weakness as had been demonstrated in cases of peripheral neuritis by Dr. Kuffler and myself at an earlier date (8). Hodes then plotted the maximal conduction velocity of the nerve to a parietic muscle against the maximal voltage of that muscle in over 50 cases. It was evident from these results that if a given muscle of one patient were stronger than the correspond-

ing muscle of another patient then the nerves supplying the former would conduct more rapidly than would those innervating the latter. Preliminary histological observations of anterior horn cells of paralyzed monkeys showed results suggesting a neural involvement dependent on axonal size.

Even with the relatively primitive electrophysiological techniques available during the early part of this century, clinical physiologists became interested in their application for the study of spinal cord reflex activity in human subjects. Hoffman (10) was among the first to demonstrate the feasibility of studies of this type. He first devised a method for recording the impact of a blow on the patellar tendon and in the same record the electro-myogram from the quadratus muscle. He realized that the measurements were complicated by the characteristics of the sensory end organ. He went a step further and found that by electrical stimulation of a mixed nerve he was able to excite a reflex response which could be recorded electrically. Thus, as was to be expected, gave a shorter reflex time 17 to 18 milliseconds for the patellar reflex and 28 to 29 for the Achilles reflex.

Dawson and Merton have recently begun studies using this type of experiment for determination of spinal reflex activity. This method should be very fruitful in the study of certain diseases of the spinal cord and also the activity of various pharmacological agents on this region of the nervous system. It is possible to stimulate one motor unit and get a reflex response in the same motor unit.

Dr. George Dawson, working at the National Hospital in London, found in studying a patient suffering from myoclonic seizures that electrical stimulation of a peripheral nerve resulted in changes of electrical potential detectable on the scalp (1). These changes in potential were largest over the hemisphere on the side opposite to that stimulated. They were located near to the midline when the lateral popliteal nerve in the leg was stimulated and more laterally when the ulnar nerve was stimulated in the arm. Extending these studies to healthy subjects, Dawson has been able to demonstrate that potential changes of cerebral origin which probably arise in the central and post-central cortex may also be detected on the scalp following electrical stimulation of peripheral nerves. In a record taken from Dawson's paper which appeared recently one can see the responses to stimulation of the left and right median nerves at the elbow and the left lateral popliteal nerve at

the head of the fibula. The electrodes were applied over the surface markings of the sensory motor area. A stimulus to the left median nerve produced a potential change maximum near the electrode on the right side 6 cm from the midline. A stimulus to the right median produced a similar disturbance on the left side of the head. Stimulation of the left lateral popliteal nerve gave rise to a potential change nearer to the midline electrode than to any of the others. The latency of the response to stimulation in the leg was 36 plus or minus 2 milliseconds which was about 14 milliseconds longer than when the median was stimulated.

Finally, I would like to discuss briefly an interesting group of clinical changes which are associated with alterations in serum potassium concentration. The introduction of the flame photometer has made it possible to study more extensively various conditions associated with changes in potassium. The following illustrates what has been accomplished by improvements in the chemical techniques. As one observes the ratio of potassium in serum water and extracellular fluid water calculated by observers over a period of years one can see that it now approaches the theoretical value expected from the Gibbs-Donnan equation (2). It now seems possible that by determinations of serum potassium level and the concentration within the muscle itself one may be able to get accurate values of the relative concentrations bathing the two sides of the cell membrane. These studies when combined with physiological measurements will find useful application in the study of patients with family periodic paralysis having low serum potassium as well as certain patients with diabetic acidosis who also exhibit hypokalemia. A clinically similar paralysis may accompany elevation of serum potassium in certain cases of uremia. It is of interest that in all three of these clinical conditions there is profound muscular weakness with diminution and loss of reflex activity, but little or no alteration in sensation. The distribution of the weakness, particularly marked in the extremities and trunk, is also the same. Preliminary studies have been made of the electro-myogram in patients with family periodic paralysis during an attack and in the normal state. It will be noted that when the electrodes are placed far apart along the muscle that the potential tends to be more monophasic in character than usual. The normal diphasicity is maintained when the electrodes are placed together in the middle third of the muscle. It has been noted that there

is very little change in the shape of the potential where the electrodes are placed 2 centimeters apart. Although there are other possible explanations this type of experiment suggests that the defect may be in the transmission of the excitatory wave along the muscle fiber itself. Similar studies are not available in the clinical states associated with elevation of serum potassium.

Thus, one can see that although the methods are still relatively crude and that the potential contributions of the clinical neurophysiologists are still in the developmental phase it is becoming possible to gather more and more reliable facts and the unique physiological experiments created by naturally occurring disease may uncover many stimulating leads.

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# AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

## Symposium on Methods for the Determination of the Purity of Substances of Biochemical Interest<sup>1</sup>

VINCENT DU VIGNEAUD, *Chairman*

### VALUE OF CHROMATOGRAPHY IN DETERMINING THE PURITY OF SUBSTANCES OF BIOCHEMICAL INTEREST

HAROLD G. CASSIDY

*From the Department of Chemistry, Yale University, New Haven, Connecticut*

In order to evaluate chromatography as a method for determining purity we must first define what we mean by purity. This will enable us to set up criteria for judging the methods which test purity. Then, after having set the standards we may evaluate the method of chromatography by seeing how effectively it meets these standards. The standards which we set up are those abstracted, as it were, from all methods of testing purity; they are the principles of the methods of testing purity.

Upon close examination of what we mean by the determination of purity we discover that what we really mean is the detection of impurity. There is no sure way of proving a substance to be pure. We can only show with any confidence that if it contains impurities of this and such a kind they cannot exceed this and such an amount. If we use crude methods of finding impurities then the degree of purity can be stated only to a crude approximation, and with progressively closer examination we become correspondingly more able to detect impurity. It is evident, therefore, that the definition of a degree of purity must be made on the basis of the operations used to detect impurity. We do not define 'purity'. We define a degree of freedom from impurity as disclosed by certain given measurements. This operational definition of purity emphasizes that the result of the measurement depends on the instruments used, and emphasizes, further, that purity is a relative matter. There is consequently no such thing as absolute purity, and any statement which purports to define an absolute purity is operationally meaningless. This has been known,

or suspected, for a long time, of course, and an excellent discussion may be found in Timmermans' *Chemical Species* (1), and in Barnes' article in a recent symposium (2). When we use the word 'purity' in the chemical sense we must remember that we mean qualified purity, degree of purity.

What, then, are the principles of the methods for determining purity? They are that in all methods of testing purity two operations are involved. All methods of testing purity involve tests of identity, and the most subtle and reliable tests of purity involve also, and usually simultaneously, attempts at separation (3). Consider, for example, the melting point. The melting point is sometimes used as a test of purity, but only as it is used to test identity. It may indicate identity of the one substance with another which *may* previously have been proven pure. For a more profound test which involves the melting point one has to use the cooling curve (4). For if a substance is pure it will pass through a fractional solidification involved in taking a cooling curve in such a way that, barring supercooling, the solidification temperature at which crystal formation first begins will be the same as that at which the last material solidifies. Thus the cooling curve, since it combines an attempt at separation (that is, fractional crystallization) with a test of identity of the putatively purified fractions (that is, freezing temperature) meets the requirements laid down for the most acute type of test of purity. Moreover, such a test of purity is sufficient to itself in that it does not, as a test of purity, require any standard or reference substance (as does the melting point).

The boiling point measurement and the Swieto-

<sup>1</sup> Atlantic City, N. J., March 1948.

slawsky ebulliometry (5) bear the same relation to each other as do the melting point determination and the cooling curve measurement, also the single solvent partition and the Craig distribution measurement (6) bear an analogous relation. The relationship is that between a single step and a countercurrent operation (3).

However, any test of purity is subject to certain limitations. A eutectic mixture behaves like a pure substance in the cooling curve apparatus, an azeotrope behaves like a pure substance in an ebulliometer, and analogous behaviors will be recognized in other separations. It therefore follows that no single test of purity is conclusive, only is the weight of evidence for purity overwhelming when several tests have been applied and found to be met. For example, the ebullioscopic measurement at several pressures is needed to exclude with certainty the existence of an azeotrope, in the case of solvent distribution, the use of several quite different solvent pairs is needed.

The question may now be put: does the chromatographic method meet the requirements for a method of testing purity (2)? In the chromatographic method a mixture to be separated is passed over an adsorbent (fig. 1). If several substances with different adsorbabilities are present, the more strongly held one will be retained near the point of application of the mixture, the less firmly held ones further along the adsorbent. Then, by developing the chromatogram, the components may be separated from each other on the basis of their adsorbabilities. This is the principle of chromatography, the various methods of its application are not of consequence here. When the method is used as a test of purity the substance to be tested is applied to the adsorbent. As the substance meets the adsorbent and becomes adsorbed to the surface, any impurity will be expected to be either more or less strongly adsorbed, so that whether on development or frontal analysis there will be evidence of more than one zone. If, however, the substance is pure, the chromatography will not separate it, and a single zone will result. That a single zone is present may be established by any of the methods of recognizing zones: concentration measurements in frontal analysis, displacement development, or elution analysis, or by any other methods which can show that one and only one zone is present in the chromatogram (7). The answer to the question must therefore be that chromatography meets the requirements for a method of testing purity,

for not only does it involve a test of identity (since different substances would be adsorbed to different degrees), but it involves an attempt at separation by virtue of the countercurrent application of the adsorption partition.

An examination of actual cases needs to be made to see whether in practice the method redeems its promise. Since not very many tests of chromatography have been made for the express purpose of detecting impurity in substances, but rather chiefly for the purpose of separating mix-

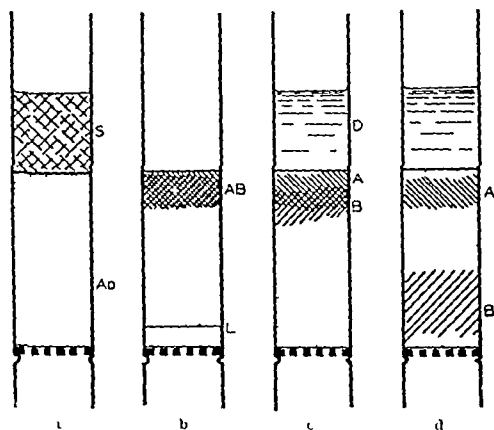


FIG. 1. Chromatography of a mixture of two solutes A and B. a. The solution containing A and B is placed above the adsorbent Ad. b. The solution has been pressed into the adsorbent, a mixed zone AB is formed, and the 'front' of pure liquid is shown at L. c. Developer liquid D is being passed into the column and development of the zones is beginning. d. Continued passage of developer liquid has produced a developed chromatogram, with the separate zones shown at A and B.

tures, an attempt will be made to derive information about the effectiveness of the chromatographic method from both classes of experiments. The literature on chromatography is voluminous, and even the papers which are directly useful to this argument are too many to deal with here. For this reason only a few which illustrate the use of the method with protein, carbohydrate and lipoidal substances will be chosen.

The use of chromatography is rather new in the field of the proteins and their degradation products, but already we have excellent evidence of its value. Thus Martin and his coworkers (8), using paper strip partition chromatography (9), examined an authentic sample of Thudichum's 'glycoleucine' and found it to be leucine. They showed, in another experiment, that not more

than 0.03 per cent of the nitrogen of hydrolyzed spinal cord could be present as *norleucine* since when 0.5  $\mu$ g of *norleucine* was added to 1.2 milligrams of hydrolysate it could be clearly recognized, but none could be detected in the hydrolysate alone. The work of Moore and Stein (10) at the Rockefeller Institute shows how very accurately the chromatographic method can analyse a protein hydrolysate, and the further sharpening of the chromatographic tool by the use of the radioactive 'pipsyl chloride' developed by Cannan and his coworkers (11) indicates that though the application of the method in the protein field is new, yet it already shows that we can expect much from the use of chromatography as a test of purity of amino acids, and, indeed, of peptides, enzymes and similar substances.

In the field of the carbohydrates we can call immediately upon the results of Wolfson and his coworkers (12). They have shown, in a series of papers emanating from Ohio State University, that, for example, 2 per cent D-mannitol can be separated from its admixture with sorbitol by chromatography on the adsorbent Florex XXX (13). They showed, also, that a mixture of approximately 0.2 milligrams each of  $\alpha$ -D-galacturonic acid, D-galactose, D-glucose, D-xylose, and L-rhamnose could be separated into five zones on the same adsorbent, using isopropyl alcohol as the solvent and developer.

Very many lipid-soluble substances have been examined chromatographically for purity. Elegant work of this kind was done by Winterstein and Stein (14) who were able to detect and separate 2 milligrams of dipalmityl ketone in 300 of a hentriacontane which showed the theoretical analysis and a good melting point. They also could concentrate 0.5 per cent ergosterol in admixture with cholesterol through chromatography on alumina. It was in this laboratory also (15) that chromatography was used to isolate 0.05 per cent carbazole from anthracene. The anthracene so obtained showed the blue fluorescence which is known to be quenched by 1/30,000 per cent of naphthacene. The original sample of anthracene had contained 0.5 per cent of naphthacene. Man (16) and his coworkers at the Bureau of Standards have shown that the aromatic hydrocarbon content of a mixture of paraffins, naphthenes and aromatics can be determined to within  $\pm 0.2$  per cent. Zechmeister and McNeely (17) showed that it was possible to determine 1 to 2 per cent of *cis*- or *trans*-stilbene in admixture with the other form.

In the fat series Kaufmann (18) showed that the purest grades of caproic, lauric, myristic, palmitic, oleic and stearic acids contained significant amounts of impurities consisting of higher and lower fatty acids. The method was found applicable also to glycerides. Cassidy (19) was able to demonstrate unequivocally the presence of 0.67 per cent ester in the presence of mixed fatty acids by chromatography on charcoal. Claesson (20) could demonstrate 3.9 per cent of lauric acid in admixture with myristic.

Ruzicka and his colleagues (21) have demonstrated, through their precise fractionations of natural mixtures, the value of chromatography in separating and testing the purity of lipid-soluble substances. As an example of the precision of their work one may cite the isolation of friedelin. From 82.2 gm of nonsaponifiable material (representing 1500 kg of pig spleen) they isolated 25.4 mg of the triterpene friedelin. This represented 0.03 per cent of the nonsaponifiable fraction, and was present as an impurity from cork stoppers into contact with which the material had come.

No discussion of the use of chromatography as a test of purity would be representative without mention of the work on plant and animal pigments. We might mention here the work of Zechmeister (7) and of Strain (7).

Chromatography shows a limitation analogous to that already described in connection with other testing methods, that is, two different substances may be adsorbed under certain conditions to the same extent, thus giving a false impression of purity. This situation is analogous to that encountered in eutectic formation and azeotropism (3). The remedy is the same as in those cases, namely, to change the conditions of the test. With adsorption this involves using different adsorbents and different solvents, and preferably choosing these to have different polarities from those first taken. An observation of Bauernfeind and coworkers (22) may supply an example of this limitation. These workers found two pigments which, as they said, were chromatographically identical by mixed adsorption on activated alumina and development with reagent chloroform, but which were not identical spectrophotometrically.

A further limitation on chromatography, and one not absent from other methods of testing purity, is in effect the reverse of this. Instead of a mixture appearing pure, a pure substance yields several zones. This is often due to some catalytic effect of the adsorbent in which a sub-

stance is altered (23). Thus a pure *cis* compound can be altered to a mixture of *cis* and *trans*, which then yields two zones. This effect can often be anticipated and corrected, fortunately it is not very common. The classical example of this effect was the observation by Gillam and El Ridi (24) that carotenes could be isomerized by chromatographic adsorption. This observation led, in the hands of Zechmeister and his students, to the remarkable and important work on the configuration of the polyene pigments (25).

That biologically important substances may be altered on the chromatographic column has

coloration, when adsorbed from solvents which contained hydrochloric acid (as would chlorinated solvents which had been standing around for some time).

Fortunately, as has been remarked above, these situations are relatively rare, and can be anticipated when very active acidic or alkaline adsorbents are being used. The appearance then of two zones would not necessarily imply impurity in the initial substance.

It must be evident from the remarks made so far that chromatography should be a very useful method for testing purity of substances provided

TABLE 1 ORDERS OF ABSTRACTION

| LEVEL OF ABSTRACTION     | LIVING   | NON LIVING   | ORDER OF INCREASING ABSTRACTION |
|--------------------------|--|--|---------------------------------|
| Macroscopic <sup>1</sup> | First Order Facts <sup>2</sup>                           |  | ↑                               |
| Microscopic              | Org anisms   | Substances (bulk)<br>(bulk compounds)<br>(bulk elements) |                                 |
| Submicroscopic           | Inferential Facts  |  |                                 |
| Molecular                | Colloids<br>Molecules and atoms<br>Protons and electrons |  |                                 |
| Subatomic                |  |  |                                 |

<sup>1</sup> By abstraction is meant a process of leaving out details (30). In passing from one level to another level of increased abstraction there are left out many details which are used to describe the first level. Thus in passing from the subatomic level to the molecular there are left out all sorts of information about nuclear construction, electron shells, electron spins, etc. These may, however, be implied in the symbol used for the molecule or atom, but they cannot be explicitly stated in the terms used for dealing with the more abstract level. This is in part the dilemma of form and function. It is the functioning of protons and electrons which makes the form of molecules. It is the functioning of molecules which makes the form of colloids and cells. There is naturally some overlapping of these levels.

<sup>2</sup> First order facts are facts which are directly observable. Inferential facts are facts which are inferred from, and to explain, first order facts.

been recognized, and, as an example, has led to the development of analytical methods which would circumvent this difficulty. For example, Haagen Smit and co-workers (26) developed a shortened method for analyzing provitamin A substances from plants in order to minimize the losses which accompanied certain methods in common use.

Many other phenomena may occur in the column on active adsorbents. As one example we may cite the observation of Tieppe (27) that triglycerides may be saponified on Brockmann alumina even with nonaqueous solvents. Tieppe also recorded that free as well as esterified cholesterol underwent catalytic decomposition on silica columns, with the development of a blue red

that they are adsorbable, and all substances show this property to some extent. Chromatography may be used to test purity of substances in the gaseous form (20), or as liquids, or in solution, and it applies to adsorption at non-mobile interfaces as well as mobile, that is, to adsorption on solids as well as to adsorption on foams and emulsions (28). Dr Craig has suggested that I mention that the chromatographic method, perhaps due to the mechanics of the arrangement, is the most *efficient* separation method we have, for a short column may provide separations which indicate it to have many hundreds of 'theoretical plates' (10, 29). When the method is used within its proper sphere it can supply a most powerful tool for testing purity. This is because it meets

the requirements which we found to be characteristic of a good method, namely, because it involves an attempt at a separation of the substance under test and at the same time a comparison of the fractions obtained to test their identity

The problem of the nature of chemical purity has aroused so much discussion that it may not be amiss to attempt here to clarify certain points involved in it. In table 1 is presented a classification of certain phenomena. The concept of chemical purity can be applied correctly *only* at the level of macroscopic and microscopic first order facts. Pure is a word with many meanings, each depending on the context within which the word is used. It is essential to distinguish the different meanings. Thus when one speaks of a pure cell-suspension the word is being used in an entirely different sense from that used when one speaks of a pure substance. In the former case one can

prove that all the cells are of one kind, in the latter it is impossible to prove that all the molecules are identical. The word pure cannot be used at the molecular or subatomic levels with any chemical meaning. There is no such thing as a chemically pure molecule (And of course, in the case of bulk elements, these are by definition pure. The word 'bulk' is used here to qualify 'element' which is often used to mean 'a molecule of an element'). Biochemists, since they are working in a borderline field, are beset with all sorts of difficulties in nomenclature. This note is written with the hope that at least one of the difficulties will be clarified. The application of the idea of levels of abstraction can clarify many other problems. An example of the 'paradoxes' which can be devised by dealing in the same terms with two levels of abstraction at once can be found in a recent paper on purity (31).

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## COUNTER-CURRENT DISTRIBUTION

LYMAN C CRAIG

*From the Rockefeller Institute for Medical Research, New York City*

The classical approach of organic chemistry to the problem of purity has always been that of fractionation. A preparation has been regarded as 'pure' if all attempts to fractionate it have failed to change its measurable properties. It should be satisfying to the organic chemists to know that this approach has recently been stated (1) by a physical chemist, Professor Eyring, to be the definition of a component in the thermodynamic sense of the phase rule. Irrespective of viewpoints, nearly all fractionation techniques are useful in purity studies.

Biochemistry deals with an enormous number of chemical compounds with widely differing properties. Some are gases, some are liquids and some are solids either crystalline or amorphous. Wide variation in stability is encountered. Obviously no single fractionation technique will be the most effective for the separation of each type. Since the determination of purity requires maximum separating power under conditions of complete stability, a choice of the most effective method must be made. Fortunately, the different methods often supplement each other and wherever possible, more than one method must be applied. Measurement of physical constants with adequate precision is taken for granted, as far as the present discussion is concerned.

In order to choose the most effective fractionating technique we must know something of the nature of the compound, in biochemistry particularly something of the stability of the solutes involved. Unquestionably many compounds of importance have such poor stability except in solution in a certain environment that they can never be crystallized or obtained free of solvent. Yet such a substance could cause a very specific biochemical response and it would therefore be of the greatest interest to know if such a response were caused by a single chemical individual or whether a collection of individuals were responsible for the effect. In such a problem counter-current distribution offers possibilities.

A few years ago a problem of this general character was encountered by us in studies dealing with synthetic antitumour drugs. Unexpected

toxicity was encountered in connection with the clinical study of a particular drug. This raised the question of purity and the chemists were asked for reliable data to prove that they were not at fault in claiming a pure preparation. At the time no satisfactory method existed for demonstrating purity since the properties of the compound did not permit the fractionation techniques available to be applied under favorable circumstances.

An approach to the problem was made through the application of extraction data. Although extraction is one of the oldest procedures of organic chemistry, it is usually applied in such a manner that results only approximate in nature are obtained. Purity studies require quantitative data. Particularly in biological circles it has been appreciated for some time that if enough cases are at hand, the results can be treated statistically and thereby greater precision achieved. Therefore, multiple extractions were performed in counter-current manner so that the steps of the binomial expansion were exactly followed (2). This permitted the mathematics of probability to be applied in the interpretation of the results (3, 4). The name 'Counter-current Distribution' was given to the process and an apparatus was devised whereby hundreds of nearly perfect individual extractions could be performed in an hour or more. The final result of the process could be most conveniently expressed graphically by plotting the fraction of solute in a given extraction cell against the serial number of the cell. This gave a pattern which with a single solute will exactly correspond to the normal curve of error as in figure 1. Figure 1 is the pattern of an actual distribution made with a sample of benzylpenicillin (5). Since the curve is an averaged or statistical result, a considerable error in a single extraction is unimportant.

The requirements in order to accomplish a normal curve of error are the following. Two phases must be found in which the solute will distribute itself in approximately equal amounts upon equilibration. The partition ratio must be satisfied at every step. The phases must separate easily. The partition isotherm must be linear, i.e.,

the partition ratio not be different with different concentrations of solute. Often the temperature must be held constant. The process is restricted to those cases where the partition ratio really is a constant. Each of the necessary factors can be

nearly identical partition ratios. If a deviation occurs as in figure 2, determination of the partition ratios of the solute in the individual tubes where deviation is shown will decide if the cause is impurity.

A gradual drift in the ratios as shown in figure 2 definitely indicates impurity. Figure 2 is a distribution with a sample of benzyl-penicillin which contains a small percentage of  $\Delta^2$ -pentenylpenicillin (5) and a little of another impurity. Experimental achievement of the curve of error indicates purity as far as the method and the particular system is concerned, though it alone is by no means conclusive evidence for purity. The result is analogous to a precise fractional distillation followed by an ebulliometric or boiling point study of each of the fractions. Or it is analogous to fractional crystallization followed by a phase rule study such as the determination of the solubility curve in the solvents used for the recrystallization. However, none of these methods absolutely exclude impurity but for practical purposes, if more than one of the procedures indicates purity or a curve of error is obtained in more than one entirely different system, then the preparation will be of high purity in by far the largest majority of cases.

With any method of fractionation, the degree to which a possible impurity can be excluded or the precision with which it can be revealed will depend upon the intrinsic separating power of the method. Separating power basically depends upon the selectivity of the method and the number of perfect transfers applied. A continuous fractionation process does not have discrete steps and must be interpreted in terms of the 'equivalent of the number of perfect stages'. Often this number is not constant. Stepwise extraction offers a process which can be made to operate at complete equilibrium and with any desired number of extractions. Assumptions are not required. The phenomenon corresponding to 'constant boiling mixtures' is eliminated by working at sufficient dilution. The constant which determines the selectivity is the partition ratio. It is of importance, therefore, to understand the full significance of the ratio.

In more than one instance the partition ratio has been spoken of as depending on 'solubility'. This would appear to be quite erroneous as far as the type of partition ratio used in this connection is concerned. Only at the point of complete saturation of the solute in each phase will the partition ratio correspond to a ratio of solubilities. At concentrations below this, constant pro-

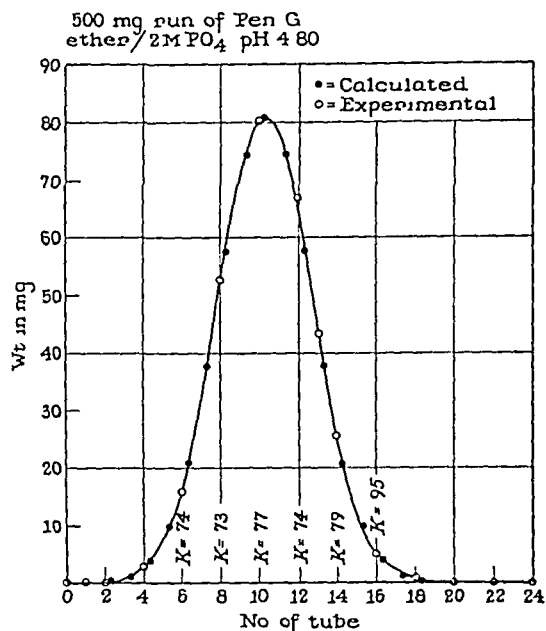


Fig 1 TWENTY-FOUR TRANSFER DISTRIBUTION of benzylpenicillin

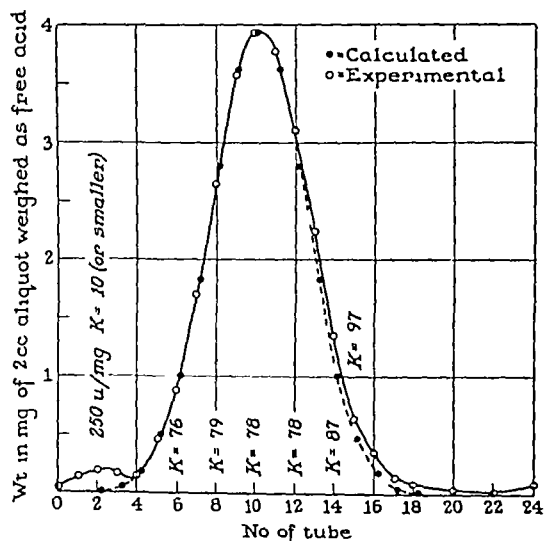


Fig 2 TWENTY-FOUR TRANSFER DISTRIBUTION of slightly impure benzylpenicillin

checked separately or a distribution can be made and from the nature of the pattern it can be derived whether or not they have been met. If the experimental distribution agrees with the normal curve of error, it follows that the necessary conditions have been met and that the solute is a single substance or more than one substance with

portionality of the activity of the solute in the two phases will seldom be found. It would seem better to consider a partition ratio as a measure of the relative attracting tendency of two phases for a solute at a specified concentration. The basis of the ratio is therefore a subtle one and usually quite different from solubility.

In the technical literature of fractionation (6) the selectivity of a particular extraction system is defined in terms of the ratio of the partition coefficients of two arbitrary solutes. This ratio is called  $\beta$  and equals  $K_1/K_2$  in the particular system. A system in which  $\beta$  is large is a selective system for separating the mixture. Obviously this should be the system of choice for demonstrating impurity if the arbitrary solutes are closely related and are similar to the one on which the purity study is to be made.

In general, those systems will be the most selective in which there is the greatest contrast in the hydrophobic-hydrophilic solvent properties of the two phases concerned. Where the solute is an acid or base, buffers are especially helpful. As regards more subtle degrees of selectivity, no general rules can be offered. The choice must be based on experimental trial.

Once the selectivity of a system has been established it is a simple matter to calculate the number of transfers required in order to detect a given impurity or to exclude it to a given percentage. Hypothetical distributions such as figure 3 bring out this point. Consider a mixture of 90% A,  $K = 1$  and 10% B,  $K = 1.2$ , i.e.,  $\beta = 1.2$ . The sum of A and B curves, the top curve, would differ sufficiently from the curve of error so that a small percentage of B in A could be detected with 100 transfers. This requires a few hours' time with our present equipment, which includes a distribution apparatus with 54 cells. Two or even three times this number of transfers are within reach. Time does not permit further discussion of this type of calculation. The picture given here is that based on weight alone and weight is certainly the most inclusive analytical method as far as unknown mixtures are concerned. However, if a more specific method of analysis is at hand which can be combined with weight, then a small percentage of impurity with a ratio of 1.1 or even 1.05 can be detected.

The reliability of the distribution curve is increased greatly by employing more than one system. Even with closely related isomers or homologs it has, in general, not been difficult to shift  $\beta$  values from 1.0 to 1.5 or more by changing systems. To be sure, optical isomers give

$\beta$  values below the experimental limit and cannot be separated or detected by the method. On the other hand, chemical combination with other optically active solutes give diastereo-isomers which often have more favorable  $\beta$  values.

The reliability of the distribution pattern is also increased by the use of several different methods of analysis for determining a curve. If the compound gives a biological response, quantitative bio assay should be used. A pure sample gives curves which are superimposable regardless of the method of analysis employed.

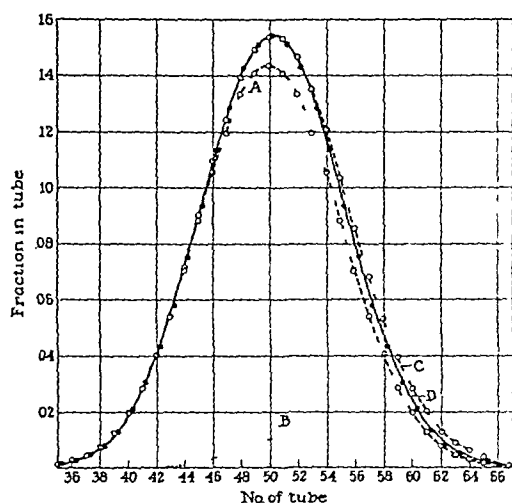


Fig 3 HYPOTHETICAL 100 TRANSFER DISTRIBUTION of a mixture of 90% compound A ( $K = 1.0$ ), and 10 % compound B ( $K = 1.2$ ) C = sum of A + B D = a curve calculated for a single substance and matched with C

If satisfactory selectivity is difficult to realize, it is possible to chemically transform the solute to another type which will offer greater selectivity. Valid data are often obtained in this way because of the quantitative nature of the distribution even in spite of inability to cause 100 per cent transformation.

One could continue with generalities indefinitely. However, in the final analysis and in spite of all the theoretical aspects, a method for studying purity must receive prime consideration if it consistently reveals impurity in preparations where other methods have failed. Our studies thus far have been satisfying in this respect. From the very beginning the method proved useful in the intimalinal field (7). Figure 4 is a representative pattern of a good antimalarial preparation, figure 5 of a poor one. Figure 6 (8) is a purity study of a sample of hexanoic acid.

Pentanoic and heptanoic acids or perhaps isoacids are revealed. Figure 7 is a pattern determined (8) on a mixture of equal parts of myristic, palmitic and stearic acids. The degree of separation and the usefulness of the method in purity studies in this series can be derived from the chart.

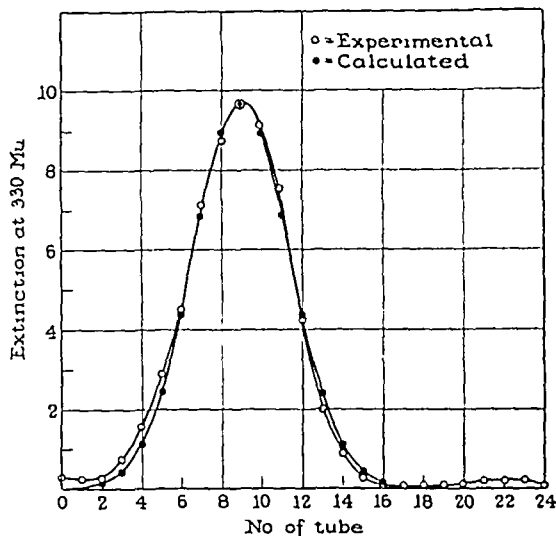


Fig 4 TWENTY-FOUR TRANSFER DISTRIBUTION of a preparation of 7-chloro-4-(1-dimethylamino-1-methylbutylamino)-quinoline di-phosphate

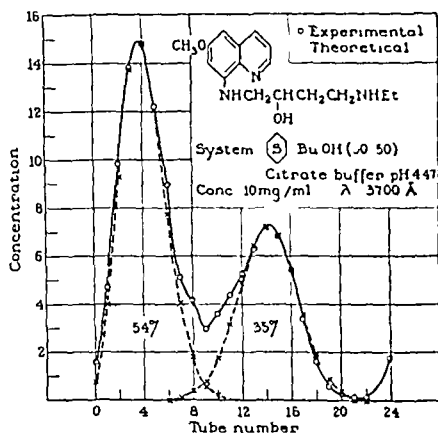


Fig 5 TWENTY-FOUR TRANSFER DISTRIBUTION of an impure antimalarial

The penicillin studies are of interest because these substances are known to be stable only under certain conditions. Yet they can be studied by distribution with adequate precision as figures 1 and 2 have shown (5, 9).

Titus and Fried (10) were able to demonstrate the occurrence of more than one streptomycin as shown in figure 8. Their result is of especial interest because of the solubility properties of the streptomycin antibiotics.

Dr. Barry, Dr. Gregory and I have recently attempted the study of antibiotics of polypeptide

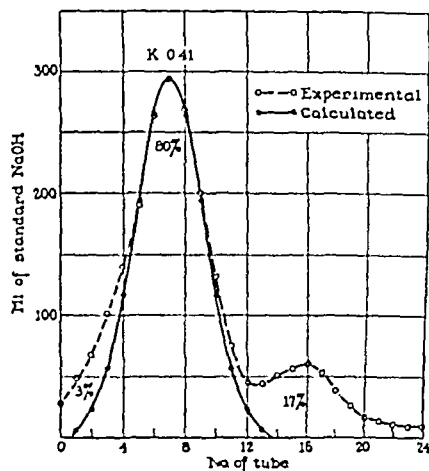


Fig 6 TWENTY-FOUR TRANSFER DISTRIBUTION of a sample of hexanoic acid

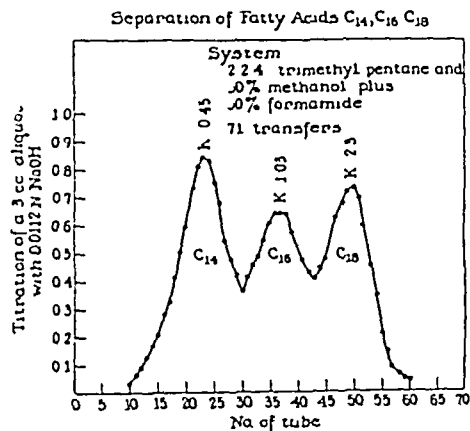


Fig 7 SEPARATION of higher fatty acids

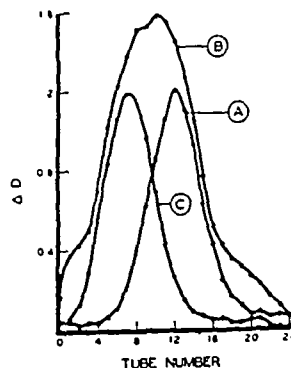


Fig 8 TWENTY-FOUR TRANSFER DISTRIBUTION of streptomycins

nature and of higher molecular weight. Significant results have been obtained with the glyamycin-tiocyidine group. The work with bacitracin and

subtilin is less advanced but here too there appears to be definite promise. Figure 9 shows a distribution of a crystalline preparation of gramicidin (11). At least four components are revealed. Three of these have now been isolated and crystallized. Crystalline components of the tyrocidine type have also been obtained. The molecular weight of these substances is believed to be in the neighborhood of 3000.

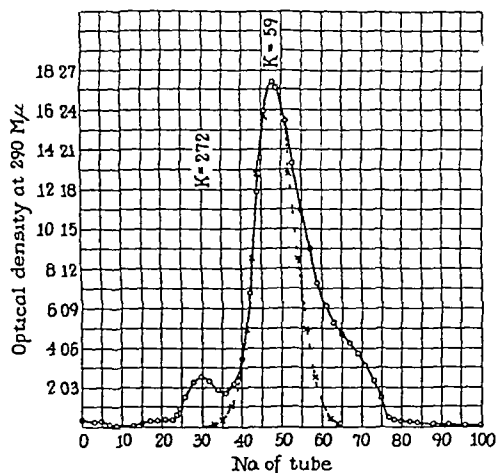


Fig. 9 ONE HUNDRED TRANSFER DISTRIBUTION of crystalline gramicidin. o = experimental, x = calculated

The work of Woolley in separating polypeptides from the partial hydrolysate of insulin, which is reported at this meeting, further demonstrates the potentialities in this field. Here bio-assay was combined with the method to good advantage.

The combination of the paper chromatographic technique of Consden, Gordon and Martin (12) with counter current distribution is particularly useful for polypeptides. Each of the fractions

from the distribution can be hydrolyzed and analyzed simultaneously for their amino acid spectrum. Shifts in composition are thus plainly revealed with amazingly little work. The starch chromatogram of Synge (13), as improved by the careful work of Moore and Stein (14), would, of course, give the quantitative picture.

Although Martin and Synge (15) considered the process they termed 'Partition Chromatography' to be mainly a liquid-liquid extraction process, a different view is now held by those of us who are closely associated at the Rockefeller Institute. We have had opportunity to study partition chromatography in direct comparison to counter-current distribution which is a true liquid-liquid extraction process. It now appears to us (mainly from the work of Moore and Stein) that the partition chromatograms can be most clearly interpreted in terms of adsorption instead of by liquid-liquid extraction. Such a viewpoint, however, removes nothing from the usefulness of that particular type of chromatography nor from the important contribution made by the English workers. Further, aside from adsorption considerations, counter-current distribution is strictly a discontinuous process operating at complete equilibrium and is thus basically different on another count.

In conclusion, the general applicability of the method may be considered. Obviously it can be applied to all solutes for which a suitable system can be found. Up to the present, systems have been found for a considerable number of bases, fatty acids, the chlorophylls, several complex polypeptides and even substances considered to be only water soluble such as streptomycin. The probability of finding systems for fats and certain sugar derivatives would appear to be good. Perhaps even some proteins are not beyond reach.

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# DIFFUSION, SEDIMENTATION AND ELECTROPHORESIS OF PROTEINS

J W WILLIAMS

*From the Department of Chemistry, University of Wisconsin, Madison, Wisconsin*

Through sedimentation velocity and diffusion studies a large amount of information about the molecular sizes of proteins (and polysaccharides) has been accumulated. In 1940 Svedberg and Pedersen (6) could write that on the basis of such data, "we know that most of the proteins in dilute solutions exist as molecules all having the same well-defined size. For several proteins this molecular size is the same even when the composition of the solution is changed within rather wide limits and is likewise independent of the method of preparing the protein."

For the determination of molecular weight by the usual sedimentation velocity method, independent studies of diffusion on the same solute must be carried out. Since the probable error in the estimated diffusion and sedimentation constants is 2 to 3 per cent, and since the partial specific volume also enters into not completely exact consideration, the molecular weight of a protein determined in this way may be in error by 5 to 10 per cent. Almost another way of saying this is to remark that with the molecular kinetic methods of the 1930's and prior 1940's we may have impurities in our protein preparations of magnitude 10 per cent and still not be able to detect their presence at all. This fact is suggestive of the need for more exact analytical tools as we approach the problem of criteria for purity of biological substances.

## DIFFUSION

For the kinetic study of homogeneity or purity of a protein the important measurement is that of diffusion constant. For this purpose it may be used alone or in some combination with sedimentation velocity or electrophoresis. Therefore, we should have before us at least the elementary theory of diffusion as it is used in the study of the physical chemistry of the proteins. The experiments have been made quite accurate in recent years by the development of the scale method of Lamm (5), and the rediscovery and improvement of the schlieren methods of Wiener (10) and of Thoenes (8) for the observation of blurring at diffusion boundaries. More recently, the quanti-

tative treatment of the interference phenomena accompanying the deflection of light by the gradients of refractive index in a freely diffusing boundary by Kegeles and Costing (4) has further increased the precision with which diffusion experiments can be evaluated.

In the more classical refractometric methods, the ordinates of the experimental curves obtained are proportional to the index of refraction gradient at each point in the cell. To calculate diffusion constants from these, use is made of a particular solution of Fick's second law,

$$\frac{\partial c}{\partial t} = \frac{D \partial^2 c}{\partial x^2} \quad [1]$$

where  $c$  = concentration,  $t$  = time and  $x$  = distance in the direction of diffusion.

For diffusion from a dilute solution into pure solvent the desired particular solution may be written as

$$\frac{dn}{dx} = \frac{n_1 - n_0}{2\sqrt{\pi Dt}} e^{-x^2/4Dt} \quad [2]$$

where  $dn/dx$  = index of refraction gradient,  $n_1$  = index of refraction of solution,  $n_0$  = index of refraction of solvent and  $D$  = diffusion constant.

In the scale method, the experimental curve is a plot of the displacement of the lines of a uniform scale photographed through the system in which diffusion is taking place as a function of the distance of these lines from an arbitrary reference line. For the calculation, the best curve is drawn through these points and traced onto another sheet with an arbitrary origin chosen for convenience. Scales of abscissae and ordinates are chosen and a table of ordinates ( $S$ ) for equal intervals on the axis of abscissae ( $s$ ) constructed. Since the origin is located by estimating the centroid in tracing the curve, a generalized form of equation 2 is used, which takes into account the distance of the true centroid from the origin

$$S = \frac{N\omega}{\sigma\sqrt{2\pi}} e^{-(s-\beta)^2\omega^2/2\sigma^2} \quad [3]$$

where  $S$  = scale-line displacement corresponding to scale-line distance  $s$  from chosen origin,  $\sigma^2 =$

$2Dt$ ,  $\beta$  = distance of chosen origin from centroid,  
 $\omega^2$  = numerical constant and  $N\omega$  = area of curve

If this analytical expression is the equation for the experimental curve, values of  $D$  may be calculated from the  $s$ ,  $S$  table by using the properties of the curve. The two most frequently used equations for  $D$  are based on the height and area (4a) and the second moment and area of the curve (4b)

$$D = \frac{(\Sigma S)^2}{S_{\max}^2} \frac{\omega^2}{2\pi t} \quad [4a]$$

$$D = \left[ \frac{\Sigma s^2 S}{\Sigma S} - \left( \frac{\Sigma s S}{\Sigma S} \right)^2 \right] \frac{\omega^2}{2t} \quad [4b]$$

Since the units of  $s$  and  $S$  in equation 3 are arbitrary, it is necessary to convert the values from different experiments to the same (dimensionless) units in order to be able to compare them. This is usually done by the transformations

$$\begin{aligned} \Xi &= \left[ \frac{5\sigma}{\omega \Sigma S} \right] S \\ \xi &= \frac{(s - \beta)\omega}{2\sigma} \end{aligned} \quad [5]$$

The resulting set of values of  $\Xi$  and  $\xi$  may then be compared with those from other experiments and with the corresponding normal (Gauss) curve

$$\Xi = \frac{5}{\sqrt{2\pi}} e^{-2\xi^2} \quad [6]$$

Coincidence of the experimental points with the normal curve shows that equation 2 applies to the diffusion system studied

The assumption that equation 2 does describe the variation of index of refraction gradient (and so of concentration gradient) as a function of distance in the diffusion cell and of time, so long as diffusion has not progressed far enough to affect concentrations at the end of the cell, has been justified by a great mass of experimental data. As a result, coincidence of the experimental with the normal curve is taken as an indication of homogeneity, or, more strictly and properly, no inference about the solute is drawn from coincidence, but deviations of the experimental curve from the normal are attributed to some peculiarity of the solute, including the presence of impurity. Thus, this method is not entirely satisfactory

Equation 2 may be solved for  $D$  to give

$$D = \frac{(n_1 - n_0)^2}{\left( \frac{dn}{dx} \right)_{\max}^2 4\pi t} \quad [7]$$

since  $dn/dx$  has its maximum value at the center of the peak where  $x = 0$ . In the Kegeles-Gosting interference method the determination of the diffusion constant is based on measurements of the positions of interference bands formed by the schlieren lens and the diffusion gradient in the plane of the knife edge. The important formula is

$$D = \frac{(n_1 - n_0)^2}{\left( \frac{C_i}{ab} \right)^2 4\pi t} \quad [8]$$

where  $C_i = \frac{Y_i}{e^{-\xi_i^2}}$  and  $Y_i$  is the downward displacement of  $i$ 'th interference fringe from the undeviated slit image in the plane of the knife edge. The quantity  $e^{-\xi_i^2}$  is obtained from tables and is a function of the number of the particular fringe and the total number of fringes in the interference pattern. Thus  $C_i$  becomes the maximum downward displacement of light in the focal plane of the schlieren lens predicted by geometrical optics and

$$\frac{C_i}{ab} = \left( \frac{dn}{dx} \right)_{\max} \quad [9]$$

The downward displacement  $Y_i$  below the undeviated slit image in terms of diffusion constant is

$$Y_i = \frac{ab(n_1 - n_0)}{2\sqrt{\pi Dt}} e^{-\xi_i^2} \quad [10]$$

A value of  $D$  from each fringe may be obtained by an accurate measurement of the values of  $Y_i$ . Since  $Y_i$  is usually of the magnitude 10 mm and may be measured to within a few microns, this method of measuring the diffusion constant of an ideally diffusing substance should be capable of another order of magnitude of accuracy as compared to that obtained from the scale line displacement methods.

Actually this is true, and as a result we are provided with a more sensitive method for the detection of the abnormalities in diffusion which are caused by the presence of impurities in a protein solute (or by concentration dependence of diffusion). In the classical Lamm scheme indication of impurity is the failure of the normalization, which as suggested above is not sensitive

In the interference method indication of impurity is lack of constancy of the maximum downward displacement  $C_1$ , as computed from successive fringes. The values ( $n_1 - n_0$ ) must be measured in an independent experiment.

It will be well to emphasize that there has been no gain in theoretical precision. The beneficial result comes from the fact that it is possible to measure the fringe displacements with one order of accuracy higher than is possible in the case of the scale-line displacements. Detailed consideration of this general problem will be the subject of a subsequent scientific contribution.

We should mention that Coulson, Cox, Ogston and Philpot (3) have just published a description of a rapid method for determining diffusion constants in solution which is based on the interference principle. The basic theory is the same as that of Kegeles and Gosting, but the English investigators have not as yet developed the theory to the point where the detection of the presence of impurities has become possible.

#### SEDIMENTATION VELOCITY

The usual sedimentation velocity criterion for the homogeneity of a protein is that a peak in the scale line displacement distance curves moves in the centrifugal field as a single apparently symmetrical boundary when the protein is dissolved in buffers of various hydrogen ion concentrations and ionic strengths. Actually, this is judgment based largely upon diffusion and, to be more precise, the investigation should be made to determine whether the boundary has been broadened solely by diffusion. This may be accomplished by comparing the diffusion curves obtained in the ultracentrifuge experiments (corrected for the sector shape of the cell) with the theoretical curves calculated from values of  $D$  found by independent diffusion experiments. If the two sets of curves coincide we have a good criterion of monodispersity—in this instance all the molecules have the same specific sedimentation constants. Unfortunately, this test is hardly to be recommended because it is difficult to maintain temperatures of sufficient constancy in a centrifuge cell so that convective disturbances can be avoided. Slight convective inhomogeneity strongly influences the apparent diffusion constant and failure of actual and theoretical curves to coincide might be misinterpreted.

Again, if the molecules are large (low diffusion constants) differences in sedimentation constant

as small as a few per cent may be readily detected in the sedimentation diagram. When, however, the molecules are small (high diffusion constants), two substances having even as high as 25 per cent difference in sedimentation constant may appear from the sedimentation diagram to be a uniform substance with sedimentation constant intermediate between that of the single components. Calculation of the apparent diffusion constant will indicate the nonhomogeneity by giving values which show a pronounced drift with time, and the average constant obtained in this way will be considerably higher than that found from special diffusion experiments.

#### ELECTROPHORESIS

Diffusion and sedimentation experiments may give information about the mass (and shape) homogeneity of proteins. A very satisfactory physical chemical test for another kind of homogeneity of a protein is found in the electrophoresis experiment of the present day. In it a quantitative measure of the charge heterogeneity may be obtained through the determination of the standard deviation of the mobility distribution, assuming that the protein has a Gaussian distribution of mobilities.

The usual criterion for the electrophoretic homogeneity of a protein is that it migrates as a single boundary in an electrical field in buffers of various hydrogen ion concentrations and ionic strengths. To this criterion must be added a second one to the effect that the rate of spreading of the protein boundary under conditions such that convection and anomalous electrical effects are avoided should be no greater than that due to diffusion alone. If this spreading is greater than can be accounted for on the basis of diffusion and the gradient sharpens on reversal of the current, the protein may be heterogeneous with respect to electrophoretic mobility even if a single symmetrical boundary is observed.

Tiselius pointed out that the reversible spreading due to lack of homogeneity of the migrating substance with regard to mobility may be distinguished from that caused by the boundary anomalies because the latter effects are always of opposite sign at the two boundaries, while in the case of inhomogeneous proteins both boundaries behave in the same manner. Tiselius and Hoisfall (9), and Sharp, Taylor, Beard and Beard (7) have proposed quantitative methods for the representation of boundary spreading, but neither



group gave sufficient attention to the actual distribution in mobility, even in one variety of protein molecules and to the superimposed diffusion

There are four factors which influence the rate at which a protein gradient will spread under the influence of the electrical field. Two of them are irreversible, two reversible. 1) Diffusion is superimposed irreversibly on electrophoresis. 2) Convections due to temperature gradients set up in the cell by electrical heating produce irreversible changes. 3) Conductivity and pH differences across the boundaries give reversible changes (which can be largely eliminated by conducting the experiment at the isoelectric point). 4) Actual electrochemical inhomogeneity of the protein molecules gives rise to reversible electrophoretic spreading (broadening of the peak due to differences in net charge or size or shape).

Sharp and collaborators (7) have shown that when diffusion during an electrophoresis spreading experiment is negligible compared to the electrical spreading, the mobility distribution may be obtained from the refractive-index gradient curves, and a heterogeneity constant ( $H$ ) may be calculated from the time rate of change of the standard deviation of the gradient,  $\frac{\Delta\sigma}{\Delta t}$ .

They write

$$H = \frac{\Delta\sigma}{\Delta t E}, \quad [11]$$

where  $E$  is the electric field strength

In the case in which diffusion of the protein during the experiment is not negligible and the mobility distribution may be represented by the Gaussian probability function, Alberty (1) has shown that the experimental refractive index gradient at the isoelectric point will have Gaussian form, and a heterogeneity constant,  $h$ , may be calculated by using equation 12

$$D^* = \frac{\sigma^2 - \sigma_0^2}{2t_E} = D + \frac{E^2 h^2}{2} t_E \quad [12]$$

In this equation  $\sigma_0$  is the standard deviation of the gradient curve at the moment the field is applied, and  $\sigma$  is the standard deviation after electrophoresis for  $t_E$  seconds. According to this equation, the 'apparent' diffusion constant,  $D^*$ , should plot as a straight line against time of electrophoresis and extrapolate back to the normal diffusion constant,  $D$ , at zero time. In order to apply this method it is necessary that all the

protein molecules in the sample have the same diffusion constant. Also, the protein must be soluble and stable at its isoelectric point. These conditions are fulfilled for the proteins studied here in Madison. The heterogeneity constant,  $h$ , is actually the standard deviation for the mobility distribution  $g(u)$

$$g(u) = \frac{1}{h\sqrt{2\pi}} e^{-u^2/2h^2} \quad [13]$$

The mobility distribution for the protein may therefore be plotted by using the value of  $h$  determined from the slope of the graph of  $D^*$  vs  $t_E$  and tabulated values for the Gaussian probability function.

As a check on the elimination of convection, the field should be reversed for an equal period of time to bring the boundary back to its initial state except for diffusion. If the field is reversed at time  $t_i$ , the apparent diffusion constant during the reversal period is given by equation 14

$$D^* = D + \frac{E^2 h^2 (2t_i - t_E)^2}{2 t_E} \quad [14]$$

According to this equation the apparent diffusion constant becomes equal to the true diffusion constant at  $t_E = 2t_i$ . Equation 14 may also be used to calculate the heterogeneity constant from data obtained during the reversal period.

A protein which shows reversible spreading in electrophoresis at the average isoelectric point must contain molecules with isoelectric points higher than the average and molecules with isoelectric points lower than the average. The isoelectric point distribution may be determined electrophoretically by studying the reversible spreading at several pH's in the isoelectric range. If the rate of change of the average mobility of the protein with pH,  $du/dpH$ , is constant in this range and the heterogeneity constant is independent of pH, the mobility distribution may be used directly to calculate the isoelectric point distribution. For instance, these conditions appear to apply very well in the cases of human  $\gamma$ -globulin and horse pseudoglobulin. The isoelectric point,  $pI$ , of molecules with a mobility  $u$  at the average isoelectric point of the protein  $pI_{av}$  is given by equation 15

$$pI = pI_{av} - \frac{u}{\frac{du}{dpH}} \quad [15]$$

Detailed electrophoresis spreading experiments have been carried out with human  $\gamma$ -globulin

immune lactoglobulin, bovine serum albumin,  $\beta$ -lactoglobulin, ovalbumin, ribonuclease, and a number of others (2) All of these materials had been carefully prepared by recognized methods and in some cases were crystalline in form With the possible exception of ribonuclease none of the proteins were found to be completely homogeneous Of course, it is not surprising that the antibody proteins among them are not homogeneous, since they contain many different antibodies

#### CONCLUSION

In this report we have emphasized that a symmetrical stationary boundary in diffusion or moving boundary in sedimentation or electrophoresis is not sufficient evidence for the homogeneity of molecularly dissolved proteins and that careful attention must be devoted to the exact mathematical form of the curve which represents the boundary We have selected as the basis for discussion two new approaches which have been developed in this Laboratory, the Kegeles-Gots

interference method for the study of diffusion and the Alberty treatment of reversible boundary spreading in electrophoresis, as being steps in the improvement of the more classical kinetic methods so that detection of heterogeneity in protein systems becomes more satisfactory

It has been our experience that a number of proteins obtained from several laboratories in the United States, reputedly of crystalline form and constant solubility, cannot now meet these new kinetic tests for homogeneity Actually, the interference method for diffusion is so sensitive that momentarily we have put aside work with it as an instrument for the characterization of proteins and have gone back to some of the purified simple sugars, simple electrolytes and amino acids for study, expecting that as the method is further developed and consolidated preparative procedures for proteins will have been improved to provide more satisfactory solutes The information which has been here collected is intended to be of aid in biochemistry

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# SOLUBILITY METHOD OF ANALYSIS

ROGER M HERRIOTT

*From the Rockefeller Institute for Medical Research, Princeton, New Jersey*

The solubility method as a test of purity has been examined in very few instances (1-3) with mixtures of two or more known components. In spite of this deficiency, the method has the following general properties to recommend it: (a) It is based on the thermodynamic principles of heterogeneous equilibria which are among the soundest of theoretical concepts in chemistry. The phase rule of Gibbs was derived by purely mathematical reasoning and is not dependent on any assumption with regard to theories of kinetics or structure of matter. (b) The method is applicable to all species of molecules. (c) The method is sufficiently sensitive to distinguish between *d* and *l* optical isomers although as an unknown a 1:1 mixture of isomers would analyze as a simple component. With some knowledge of the nature of the unknown, special solubility experiments would reveal the two component nature of even this system. (d) The equipment necessary is very simple and is probably present in every laboratory. (e) The quantity of material may be small for the limiting factor is usually the method of analysis. (f) If the material under test is impure, the solubility diagram indicates a method of separating the components, and finally, (g) Solubility experiments can be designed which with a minimum of effort will answer otherwise difficult problems such as the identity of synthetic and natural occurring products or distinguish between a mixture of optical isomers and a racemic compound.

On the debit side there are a few systems of two components which as an unknown would analyze as a single component. Examples of these are a 1:1 mixture of *d* and *l* optical isomers, and Webb (4) has pointed out that isotopic species will be indistinguishable by this method.

Details of the theory and practice of the solubility method have been amply discussed (1, 5, 9-11), so that only those aspects which are necessary to the discussion or need emphasizing will be considered.

## METHOD

Briefly the solubility method consists of a) mixing various quantities of material under in-

vestigation with equal or known volumes of solvent until equilibrium is attained, b) separation of the solid phase from the solutions, c) determination of the concentration of the material dissolved in the various aliquots, and d) plotting the concentration of dissolved material against the total (solid and dissolved) per unit volume.

## INTERPRETATION OF RESULTS

A plotting of the results of the solubility data will take the form of one of the three general type curves shown in figure 1.

In those aliquots containing no solid phase at equilibrium, the dissolved concentration equals the total added and therefore the points fall on the line *OA*. This line bears at an angle of 45°C from either coordinate when the scale units are equal. As the total concentration is increased a point will be reached where a small amount of solid will remain after equilibration. The manner in which the concentration of dissolved material varies with increasing total material beyond that point where the first solid phase persists is the crux of the solubility method. The solubility may vary in any of three ways: 1) It may remain constant as represented in figure 1 by curve *OAE*. Such a curve represents a *constant solubility* and is obtained when the material is a) a single component (i.e., pure), b) a solid solution of two or more materials having identical solubilities, and c) a mixture of two or more materials which are present in the preparation in direct proportion to their solubilities. Cases *b* and *c* are not often encountered and may be detected by performing the solubility determination in solvents of a different nature or possibly by varying the temperature. In general the ratio of the solubilities of materials varies with temperature and the nature of the solvent. If the material under study has a constant solubility in several different solvents, it is probably a single component. A racemic 1:1 mixture of *d* and *l* isomers or a mixture of isotopic isomers would behave as a single component even in several solvents. With information as to the nature of the material the optically active isomers could in some instances be resolved by the classi-

cal method based on the difference in solubility of the complex salts formed with an optically active reagent. Use of a solution saturated with respect to one of the antipods as solvent would also bring out the two-component nature of even the 1:1 mixture. In this case the solid phase at equilibrium would be rich in that antipod used to saturate the solvent.

2) The solubility may increase linearly as the total increases and change abruptly to a different linear positive slope or to one of zero. This type result is obtained when the components form simple mixtures in the solid and then solubilities are independent of one another. Each time there is an abrupt change in slope a new solid phase appears. Curve *OBCE* represents such a system with pure solid phases appearing at *B* and *C*. A mixture of *d* and *l* optical isomers in any proportion other than 1:1 would be expected to have a curve of this type.

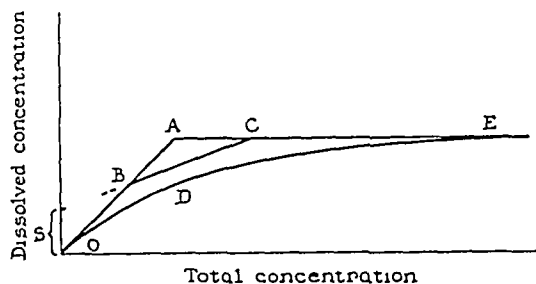


Fig. 1. GENERAL TYPES of solubility curves

Northrop and Kunitz (5, 1) have shown that the value of the intercept of the line *BC* with the ordinate is the solubility *S* of the pure component which first appears as a solid phase. The quantity of this pure component, as a fractional part of the total, is given by one minus the slope of the line *BC*. The corresponding values of the second component are obtained by difference. Kunitz and Northrop (1) obtained gratifying confirmation of their predictions upon mixing relatively pure preparations of two proteins. More recently Thorp (3) has similarly found mixtures of known isomers of *DDT* to respond quantitatively to this method of analysis.

(3) Third and finally the solubility may vary continuously as the total is increased (see curve *ODE*) approaching asymptotically a constant value. Such a result is obtained with solid solutions. In such cases no quantitative predictions are possible. If one assumes that Raoult's law holds even qualitatively, it may be predicted

that the first precipitate to appear along this curve is richer than the starting material with respect to the more insoluble component. Similarly the solution in the presence of an excess of solid is richer with respect to the more soluble component.

The behavior of a solid solution is similar to that of a system in which one of the solute components is a liquid of limited solubility but in which the second solute component is soluble. Such a system conforms to the laws of partition or distribution.

**Solubility method and the phase rule.** The phase rule is a useful generalization of heterogeneous equilibrium relating the number of components, phases and degrees of freedom. Use has been made of it to establish the solubility method on sound theoretical principles. However, it is not necessary to apply the phase rule or even understand it to make considerable use of the solubility method. Most of the work with this method is for the purpose of deciding whether a preparation consists of one or more components. The simple concept that an equilibrium condition is independent of the quantities of any of the phases will cover most of such cases, for if the solubility value is independent of the quantity of solid phase, it is a *constant solubility*, and in general this is the characteristic of a single component.

It may be worth pointing out that the phase rule is not concerned with the time required to get to equilibrium, nor does it indicate that the solute in solution is identical with that in the solid phase. In many instances they are known not to be identical.

A reversibly dissociable solute does not present a different problem in the solubility method. In such a system there is only one more component even if the solute dissociates into two parts for the concentration of the undissociated and one part will define the concentration of the third. With this one more component there will be one more degree of freedom. The concentration of the dissociated components is usually fixed by the concentration of undissociated material and the particular solvent used. Thus a dissociation or any similar reversible phenomenon does not jeopardize the usefulness of the solubility method by limiting it to nondissociating systems or requiring that the existence of such dissociation be known. If the dissociation is not reversible, then an equilibrium cannot be established and the method is not applicable.

For those interested in a phase rule proof of the method, the following is a brief description. This rule in its abbreviated form is  $P + F = C + 2$  where  $P$  = the number of phases (physically separable portions),  $F$  = the number of degrees of freedom (usually temperature, pressure and concentration of components) and  $C$  = the number of components (the smallest number of independent constituents which will define the system).

In solubility experiments the temperature and pressure, two degrees of freedom, are held constant so the equation simplifies to  $P + F' = C$  where  $F' = F - 2$ . Table 1 shows how these fac-

dry weight of solute has a very wide range of usefulness and can be a very precise measurement. Other properties must be measured when volatile solutes are under study or when concentrated salt solutions are employed as solvents. Analyses of several physical and chemical properties, specific and nonspecific, can often be rewarding, especially when examining an unknown material having specific biological activity.

The recent publication by Webb (3) that 10 per cent *l*-leucine in the presence of 90 per cent *dl* leucine resulted in a slope of only 1 per cent serves as a warning against complete reliance being placed on the method. It must follow from this result of Webb that this system is not a simple mixture of two components. Either there is solid solution formed or an additional component. In general the solubility method is open to the same difficulties and criticisms as the melting point determination to which the solubility method is related. These difficulties, however, have not prevented the melting point determination from being an extremely useful criterion of purity.

#### ADDITIVE NATURE OF SOLUBILITY AND ITS USE IN SPECIAL CASES

Special experiments based on the fact that in general solubility is an additive property have been used with marked success in answering problems concerning the identity of two preparations. Even closely related proteins or *d* and *l* optical isomers can be distinguished in this way.

Landsteiner and Heidelberger (6) showed that hemoglobins from closely related species were not identical since the solubility of the two together was greater than either by itself. Similarly Northrop (7) found that whereas the enzymatic activity, crystalline form, optical rotation, diffusion constant, and even the solubility of crystalline bovine and swine pepsins were not significantly different, a solution saturated with swine pepsin dissolved bovine pepsin. Similar procedures were used by Loing and du Vigneaud (2) to show that a preparation of 'stone' cystine was the same as hair cystine and that a racemic mixture of *d* and *l* cystine was easily distinguished from the racemic compound. The writer (8) has recently made use of this principle to show the probable identity of synthetic *dl*-3 moniodotyrosine and a product isolated from iodinated pepsin.

TABLE 1

| SYSTEM   | REGION OF CURVES IN FIG 1 | NUMBER OF |           |         |           |
|--|---------------------------|-----------|-----------|---------|-----------|
|  |                           | P         | F         | C       |           |
|  |                           |           |           | solvent | solutes   |
| Solution (no solid)                                | OB                        | 1         | 1 or more | 1       | 1 or more |
| Single pure solid in a saturated solution          | AE                        | 2         | 0         | 1       | 1         |
| Single pure solid in solution of two components    | BC                        | 2         | 1         | 1       | 2         |
| Mixture of two pure solids in a saturated solution | CE                        | 3         | 0         | 1       | 2         |
| Solid solution and corresponding solvent solutions | ODE                       | 2         | 1 or more | 1       | 2 or more |

tors vary in the different solubility diagrams of figure 1.

#### QUANTITATIVE LIMITATIONS OF THE METHOD

The limit of resolving power of this method is dependent on a) the relationship of the components in the solid phase, b) the solubilities of the individual components and c) the precision of the analytical method. If the solid phase is a solid solution, no quantitative analysis of the curve is possible. In general the greater the difference in solubility of the individual components, the greater will be the resolution. No one analytical method can be used with all materials but the

# SEPARATION OF COMPONENTS BY METHODS BASED ON THE SOLUBILITY DIAGRAM

It follows from the discussion in the section on 'Interpretation of Results' that if the system under examination is shown by the solubility method to consist of two or more components, that a procedure for their separation based on this diagram can be developed. Thus if the components are simple mixtures (case II), the first solid phase to appear, represented in figure 1 by the region enclosed in the triangle *ABC*, will be a pure component having the solubility *S* indicated in figure 1. If the components form solid solutions (case III) and their behavior does not deviate qualitatively from that predicted by Raoult's law, the first solid phase to persist at equilibrium will be richer in the least soluble component than the starting material while the solution in equilibrium with an excess of solid phase will be richer with respect to the most soluble component.

Diphtheria antitoxin (12) and swine pepsin (13) preparations whose solubility curves showed a high degree of inhomogeneity were purified by procedures based on the last of the above-mentioned suggestions. The procedures consisted of extracting the materials under conditions which dissolved only a fraction of the total, saving the solution and, after precipitating out the protein, repeating the extraction procedure on this last precipitate. With both proteins the solubility curves improved (i.e., approached more nearly that of a single component) with each extraction, and became indistinguishable from that of a single component after the third extraction.

## GENERAL PRECAUTIONS

The solvent and conditions should be chosen so there is a minimum destruction or permanent alteration of the solute during the examination. A mixed or split solvent (two miscible liquids) is often useful in adjusting the solubility of the

solute to a value which fits the needs of the particular experiment.

In general a state of equilibrium may be considered as established if approaching the same conditions from the under saturated and supersaturated sides results in the same solubility. In each experiment the solubility value must be determined at two intervals of time for the time of equilibration cannot be predicted with certainty. In those tubes containing very little solid phase more time is required to saturate than in those containing a large excess. If too long an equilibration is permitted secondary reactions may develop. In this connection the experiments of Loring and du Vigneaud (2) are of interest. In their work a mixture of *d* and *l* cystines dissolved in water forming a solution saturated to these components, and the solubility value remained unchanged for 10 to 20 hours. After this time the solubility decreased to about a quarter of the previous value. This low value agreed with that found for the racemic compound *dl* cystine. The authors also stated that microscopic examination of the solid phase showed that it was all converted to the racemic compound.

Solutions saturated with amorphous protein are of course supersaturated with respect to the crystals and if the latter begin to form the solubility will decrease. These examples merely serve to illustrate how a true equilibrium may exist for only a relatively short time.

## SUMMARY

The solubility method is a theoretically sound, highly selective method of detecting the presence of impurities. It may be used with any kind of molecule and in general can be made as accurate as the method of analysis. No special apparatus is required. When the results indicate the presence of a second component, it is possible to separate one and in some instances two components into single component fractions.

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# Symposium on Hemin Pigments and Chromoproteins<sup>1</sup>

DAVID L DRABKIN, *Chairman*

## DISTRIBUTION AND METABOLIC ASPECTS OF DERIVATIVES OF IRON PROTOPORPHYRIN (HEMIN)

DAVID L DRABKIN

*Department of Physiological Chemistry, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*

Hemoglobin, as a prototype of naturally occurring pigments and the mammalian body's most abundant specialized chemical, is a complex of porphyrin, iron and protein. Appropriately to its three fold character the investigative attack upon this compound and its natural or laboratory relatives has been multiple. Except to disclose details, resort to artifice has been unnecessary to 'label' hemoglobin, endowed with a distinctive flag, iron protoporphyrin, which, at half-mast or full, can best be seen with a spectroscope. But, this is by no means the only applicable physical probe, for hemoglobin has been studied with a greater variety of methods than any other protein. Gasometry (1), potentiometry (2), spectrophotometry (3), measurement of paramagnetic susceptibility (4), behavior in a gravitational field (5) and classical (6) as well as x-ray crystallography (7) have contributed their share to present total information.

Step by step the organic structure has been elucidated (8)—of the porphyrins, of the metalloporphyrins, and of the latter combined with nitrogenous bases—until a near synthesis or reconstitution of the grand whole, hemoglobin, has been accomplished, when 'native' globin was available (9). The proteins of the complexes have been dissected into their constituent amino acids (10), their relatively basic character established. Yet, the protein of hemoglobin has jealously guarded two conjoined secrets—how it protects the iron from rusting, and how it participates in the union with oxygen. Among complexes of iron, ferrohemeoglobin remains unique, *its iron does not rust on exposure to molecular oxygen and moisture*, but, without change in valence, combines stoichiometrically and reversibly with the gas in a process of oxygenation deoxygenation,

the basis of its biological function. In contrast, ferrocytochrome *c* oxidase is *autooxidized to the ferric state* (and at low pressures of molecular oxygen). In this process oxygen is left as a carcass, the oxygen ion, eventually to be carted off, and the aerobic spark is supplied to initiate the utilization of the energy inherent in metabolites. Thus, it is remarkable that nature has assigned to derivatives of iron porphyrin the major roles of both transport and utilization of oxygen, though the know-how of oxygenation is restricted to the hemoglobins.

*Cells need energy.* In this modern age the slogan 'need for oxygen' has given way to the more fundamental 'need for energy'. Oxygen functions to 'spark-plug' the energy-yielding processes in aerobic cells, and the 'respiratory' chemicals, hemoglobin, myoglobin, cytochrome *c*, etc., are only agents in a broader process which cuts through every segment of biological organization. An attempt has been made in figure 1 to depict in outline the biological plan. Certain features may be pointed out: *a)* There are two main integrated areas of oxygen transport and of oxygen utilization. The former is poised to resist oxidation, the latter to favor it. *b)* Regardless of whether biological enzyme systems may be made to work in a cell free medium, *in vivo* we deal with organized units, the cells, whose membrane barriers offer advantages and impose limitations, which at times may be striking. *c)* Some of the hazards, besides cytolytic agents, to which these biological processes may be exposed are illustrated in the figure by an indication of the locus of action of various histotoxic agents, CO, CN<sup>-</sup>, F<sup>-</sup>, iodoacetate, barbiturates and certain oxidants. To be effective, these substances must be able to penetrate the cellular membranes. *d)* More than 90 per cent of the effective free energy for cellular work is derived from the process,

<sup>1</sup> Atlantic City, N. J., March 1948

whose overall accomplishment is the oxidation of hydrogen in metabolites, yielding  $H_2O$ . The relationship of respiratory  $CO_2$  to  $O_2$  is no longer, as it was for over a century, the simple matter of the oxidation of carbon. Certain aspects of the various processes will be discussed.

**Distribution and natural occurrence** We have been gathering information on the total quantities of the chromoproteins in different species (11, 12). Table 1 is illustrative of the results

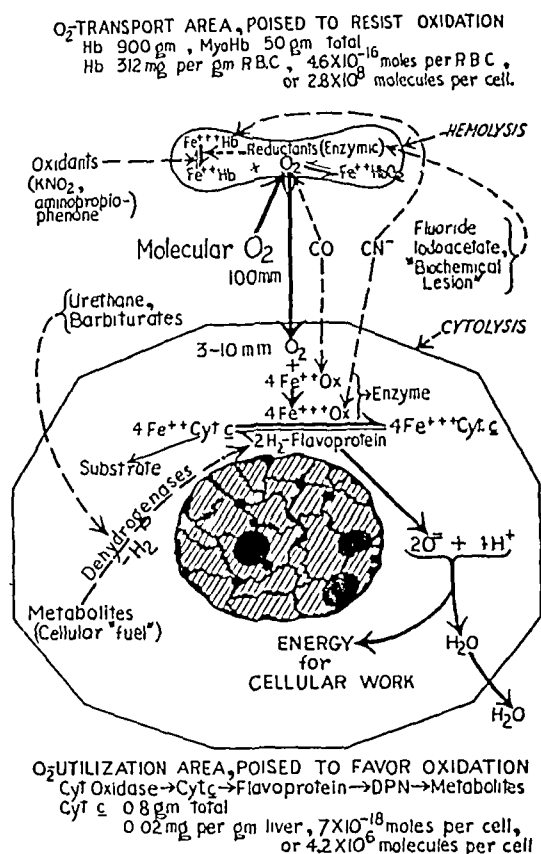


Fig 1 SCHEMA OF THE BIOLOGICAL PLAN of integration of the areas of oxygen transport and oxygen utilization. The red blood corpuscle, above, and liver cell, below, are drawn to represent relative size

The values for the quantity of hemoglobin in the species studied appear to be more or less directly proportional to the body mass. Myoglobin is particularly high in the muscles of the horse, a hard-working animal, and low in the muscles of man. Comment is unnecessary, except that this fits the concept of myoglobin as a chemical oxygen storer, or transporter "in time instead of space" (13).

The most interesting findings are for total cytochrome c, involved in the utilization of oxy-

gen. The quantitative estimation of this pigment was by means of a direct microspectrophotometric technique, developed in our laboratory (14). If rat and man only are considered, a very good case is made out for the identity of the cytochrome c values, when expressed on the basis of  $1 m^2$  of surface area, or of a fractional exponent of the body weight. We shall not quibble concerning academic arguments (15) as to which of the above bases of reference is the more correct, for insistence upon this may steer us away from a possibly significant, fundamental relationship. On the other hand, the horse is exceptional. This species has too much cytochrome c, and, thereby damages the hope for a generalization. However, the exception may be instructive. There is a philosophical exit from this dilemma, which may be worth considering. It involves, among other matters, an extension of the concept of 'basal metabolism', which unfortunately has carried with it the idea of 'fixity'. The utilization of oxygen and the need for cytochrome c function will increase with work, and the body content of cytochrome c may be related to a 'working metabolism', or to a metabolic 'capacity', which expresses the degree of expansibility of metabolism (probably under complicated hormonal as well as other control) from a basal level to a severe work level. In the horse the working metabolism probably reaches higher levels than in the other species. With this in view we were led to look into the cytochrome c content of the placid cow in contrast with the excitable, hard-working horse. While our data on the bovine species are limited, the cow fits the generalization of proportionality of the cytochrome c content to a fractional exponent of the body weight. In view of the findings, I believe that in rat, man and cow, the ratio of the working to the basal metabolism must be the same. This metabolic relationship of cytochrome c, though imperfect, appears to be one of the most striking, thus far uncovered, for a cellular chemical component.

Also of interest are the relative quantities of the three chromoproteins. In a 250 gm rat, hemoglobin myoglobin cytochrome c = 222 7 1, in a 70-kgm man and in a 500 kg horse, the respective ratios are 1150 51 1 and 359 112 1 (from data in table 1). Quantitatively, the predominant position of hemoglobin, the oxygen transport chemical, is apparent. The transportation system appears to be as important biologically in mammals as it is in modern, social organization. Not obvious in the above values,



however, is the concentration per functioning cell. In both rat and man, the concentration of hemoglobin per gm of erythrocytes is about 312 mg (16) (fig 1). The larger liver cells contain, respectively, approximately only 0.2 and 0.02 mg of cytochrome *c* per gm. When these very discrepant values are reduced to molecular magnitudes, i.e., either to moles per cell or to molecules per cell (17), a rather amazing result is obtained. Hemoglobin in rat and man =  $2.8 \times 10^8$  molecules per cell, cytochrome *c* in rat =  $4.2 \times 10^7$  molecules per cell (a value of the same

the mammals, hemoglobin, myoglobin and the cytochrome pigments are essentially intracellular. This again stresses the thought of *non-fixity*. I do not wish to alarm you, but during this symposium (duration 2.5 hours), on the basis of the rate of bile pigment production (18), it may be calculated that each of you will destroy approximately 28,000,000,000 red blood corpuscles. However, it is a relief to know that you will destroy mainly your very old, decrepit cells, those about four months of age (19). During this same interval of 2.5 hours, most of you fortunately,

TABLE 1 RELATIONSHIPS OF TOTAL CHROMOPROTEINS TO BODY MASS, *W*, AND SURFACE AREA, *S*

| SPECIES   | CHROMOPROTEIN                    | CHROMOPROTEIN |          |                      |                       |
|---|----------------------------------|---------------|----------|----------------------|-----------------------|
|   |                                  | Total         | Per kilo | Per 1 m <sup>2</sup> | Per W <sup>0.75</sup> |
|   |                                  | gm            | gm       | gm                   | gm                    |
| Rat (11)<br><i>W</i> = 0.250 kilos<br><i>S</i> = 0.0361 m <sup>2</sup>            | Hemoglobin                       | 3.19          | 12.76    | 88.3                 | 9.01                  |
|   | Myoglobin                        | 0.101         | 0.404    | 2.8                  | 0.286                 |
|   | Cytochrome <i>c</i> <sup>1</sup> | 0.0144        | 0.056    | 0.399                | 0.041                 |
| Man (12)<br><i>W</i> = 70 kilos<br><i>S</i> = 1.87 m <sup>2</sup>                 | Hemoglobin                       | 900.0         | 12.85    | 481.3                | 37.2                  |
|   | Myoglobin                        | 40.0          | 0.57     | 21.4                 | 1.65                  |
|   | Cytochrome <i>c</i> <sup>1</sup> | 0.780         | 0.011    | 0.417                | 0.032                 |
| Horse (12)<br><i>W</i> = 500 kilos<br><i>S</i> = 6.62 m <sup>2</sup>              | Hemoglobin                       | 5800.0        | 11.60    | 876.0                | 55.4                  |
|   | Myoglobin                        | 1868.0        | 3.74     | 282.0                | 17.83                 |
|   | Cytochrome <i>c</i> <sup>2</sup> | 16.6          | 0.033    | 2.51                 | 0.159                 |
| Horse (12) <sup>3</sup><br><i>W</i> = 455 kilos<br><i>S</i> = 6.23 m <sup>2</sup> | Myoglobin                        | 1347.0        | 2.96     | 216.0                | 13.7                  |
|   | Cytochrome <i>c</i>              | 24.4          | 0.054    | 3.92                 | 0.248                 |
| Cow, heifer (12)<br><i>W</i> = 182 kilos<br><i>S</i> = 2.78 m <sup>2</sup>        | Hemoglobin                       | 2215.0        | 12.17    | 797.0                | 44.8                  |
|   | Myoglobin                        | 307.0         | 1.69     | 110.3                | 6.20                  |
|   | Cytochrome <i>c</i> <sup>2</sup> | 1.24          | 0.0068   | 0.446                | 0.025                 |

<sup>1</sup> Values based on complete analyses of individual organs

<sup>2</sup> Values based on (total muscle cytochrome *c*)/0.8

<sup>3</sup> Steeplechase thoroughbred, out of training, had been insured for \$75,000

magnitude as that of hemoglobin), cytochrome *c* in man =  $4.2 \times 10^6$  molecules per cell. Operating in this, at first sight, puzzling situation are the simple factors of relative molecular and cell sizes. Hemoglobin is 5 times larger than cytochrome *c* and the liver cell some 47 times larger than the erythrocyte. We must, therefore, not be misguided by the apparently very low concentration of cytochrome *c*, when expressed in the language of the exchange counter. The functional, cellular metabolic concentration is actually appreciable, even judged by the standard of the 'highly concentrated' hemoglobin.

*Intracellular position of heme derivatives.* In

will each produce 28,000,000,000 new, mature, functional erythrocytes. Hence, whatever else you may gain, you should leave this meeting rejuvenated to some extent. On the other hand, this aspect alone of rejuvenation is a metabolic process of large magnitude, involving, in an individual adult per day, the probable conservation and reworking of some 8 gm of specialized protein (about one-fifth of the total protein requirement), the conservation and reuse of some 0.03 gm of Fe (at least three times the normal nutritional requirement) and the discarding (elimination as bile pigments) and manufacture *de novo* of about 0.3 gm of protoporphyrin.

The advantages of cellular confinement become apparent when examined in the light of pathological phenomena such as the *hemoglobinurias* and *met- or ferrihemoglobinemias*. Hemoglobin, myoglobin, and cytochrome *c* were not designed for extracellular residence, and, when these substances beyond certain amounts enter or are placed into the plasma, they can, in spite of their relatively large size (molecular weight), pass into the urine.

In our original experiments (20), hemoglobinuria was studied in the dog, following the intravenous injection of dog hemoglobin. By means of spectrophotometry, the character of the pigment excreted was definitely established, and the blood plasma levels ('threshold') beyond which urinary spillage occurred were determined accurately. The rates of excretion were similar for oxy-, met- and cyanmethemoglobin (20). Oxyhemoglobin was excreted as such, but changed rapidly in the samples obtained by catheterization into a pigment identified as methemoglobin. *Oxidants of hemoglobin are thus present in normal urine*.

This work has been extended (12) to chromoproteinuria in general by a study in the same animal, the dog, of the behavior of intravenously injected horse hemoglobin, horse myoglobin and horse cytochrome *c*, with the respective molecular weights of 66,800, 16,700 and 13,000. The excretion of injected horse hemoglobin and myoglobin was measured also in the horse, while studies are in progress as to the urinary excretion of parenterally administered cytochrome *c* in man. Myoglobinuria in man had become of interest in World War II by its association with the 'crush syndrome' (21), while therapeutic claims had been made (22) for cytochrome *c* in advance of information as to its metabolism. However, a more cogent reason for the investigation was a curiosity aroused by the obvious question of the influence of the size of the molecules upon their penetration of the kidney membrane barriers. A rather subtle and more interesting question was also involved. In the case of hemoglobin no information was available upon its molecular magnitude in the environment of the plasma. Disaggregation—a not wholly theoretical possibility (23)—could have occurred, and it could be argued that the smaller molecules, or only those molecules which had become smaller in the plasma, had leaked through the glomerular filter. The filtration of appreciably larger amounts of myoglobin, a protein very similar in type but

only one-fourth the size of hemoglobin, would therefore, be at least presumptive evidence that in hemoglobinuria we are not dealing with an altered molecular species. This very result has been obtained (table 2). As is also evident from the data summarized in this table, the dog, horse and man have approximately the same urinary spillage levels (in plasma) for hemoglobin. Whether these plasma levels may be described as *urinary thresholds* is debatable.

Attention may also be directed to the remarkable urinary excretion of myoglobin in a case of 'Monday Morning Disease', which is not quite what you may think. This is a picturesque and descriptive name of an equine disease, often fatal, which is more formally designated *Paralytic Myoglobinuria*. From the myoglobin excretion in this animal (table 2) and from analyses of the pathological muscle in which a reduction in myoglobin as well as cytochrome *c* content was found, it was calculated that at least 10 per cent of the total muscle mass had been affected. I propose that Monday Morning Disease, while of different etiology, possesses many resemblances to the crush syndrome in man. In the horse, the huge muscles of the hind limbs may be said to be 'crushed' by spastic contracture. Hence, this disease of the horse has more than economic interest for man and is an example of the valuable material available in the veterinary clinic.

Not shown in table 2 are several additional observations of interest. a) The concentration of each of the chromoproteins in the first plasma, removed at 5 to 15 minutes after their intravenous injection, was 25 to 45 per cent less than that calculated from the amount injected and the plasma volume (table 2). This early disappearance of the injected chromoprotein from plasma has not been satisfactorily explained, though such factors as blood dilution and rapid distribution over a space larger than the plasma may contribute towards the phenomenon. b) Within 2 to 6 hours the chromoproteins were totally removed from the plasma, although (in the case of the injection of hemoglobin in the dog) blood dilution persisted for more than 24 hours. c) The amount of urinary chromoprotein was proportional to the amount injected, but, more important, it was an indirect function of the amount which had disappeared from the plasma very soon after intravenous administration (see above). d) If the evaluation of chromoprotein 'cleared' from the plasma by way of the urine is based on the total amount in the plasma,

calculated from the concentration in first plasma sample (table 2), rather than from the amount injected, the 'clearance' of hemoglobin is appreciable, while myoglobin 'clearance' is virtually complete. This suggests that myoglobin, which has leaked through the glomerular filter, has not been reabsorbed in tubules, and raises doubts concerning the proposal (24) that hemoglobin (a molecule four times larger) can be reabsorbed by the tubular apparatus, at least by any of the commonly postulated mechanisms. This is one

*Ferrihemoglobin reductive function of erythrocytes* We have been discussing the usefulness of the erythrocytes in keeping the hemoglobin 'in'. This may be regarded as an essential but passive function. A study of the reversion of ferri- or methemoglobin, MHb, to ferrohemoglobin, Hb, and, particularly, of the inhibition of this process (25) have disclosed an active functional mechanism in the so called 'dead' red blood cells (17). Providentially, the erythrocytes consume very little oxygen for their own needs (17), but they

TABLE 2 CHROMOPROTEINURIA, FOLLOWING INJECTION OF HEMOGLOBIN, Hb<sub>4</sub>, MYOGLOBIN, Hb AND CYTOCHROME c, C

W = body mass in kilos, V = plasma volume in ml

| SPECIES   | CHROMOPROTEIN INJECTED             | AMOUNT INJECTED | CHROMOPROTEIN       |                                |                                | PERCENTAGE OF CHROMOPROTEIN EXCRETED |
|---|------------------------------------|-----------------|---------------------|--------------------------------|--------------------------------|--------------------------------------|
|   |                                    |                 | First plasma sample | Lowest spillage level (plasma) | Highest concentration in urine |                                      |
|   |                                    | mM              | mM/l                | mM/l                           | mM/l                           | %                                    |
| Dog (20)<br>W = 12.8<br>V = 512                               | Dog Hb <sub>4</sub>                | 0.1365          | 0.1632              | 0.0925                         | 0.3785                         | 7.3                                  |
| Dog (20)<br>W = 14.0<br>V = 560                               | Dog Hb <sub>4</sub>                | 0.3383          | 0.4110              | 0.0529                         | 1.175                          | 33.6                                 |
| Dog (12)<br>W = 8.7<br>V = 348                                | Horse Hb <sub>4</sub> <sup>1</sup> | 0.0291          | 0.0683              | 0.0320                         | 0.1340                         | 4.2                                  |
|   | " Hb <sup>1</sup>                  | 0.0109          | 0.0231              | 0.0025                         | 3.890                          | 70.3                                 |
|   | " C                                | 0.0344          | 0.0560              | 0.0014                         | 1.868                          | 48.7                                 |
| Horse (12)<br>W = 400<br>V = 23,400 (Monday Morning Disease)* | Horse Hb <sub>4</sub> <sup>1</sup> | 6.160           | 0.1892              | 0.1432                         | 0.3442                         | 0.3                                  |
|   | " Hb <sup>1</sup>                  | 0.1103          | 0.0029              | 0.0006                         | 0.1140                         | 43.0                                 |
|   |                                    |                 |                     | 0.0027                         | 1.293 <sup>2</sup>             |                                      |
| Man (12)<br>W = 50<br>V = 2250                                | Human Hb <sub>4</sub> <sup>1</sup> | 0.419           | 0.1328              | 0.0780                         | 0.4770                         | 9.7                                  |
|   | Horse and cow C                    | 0.113           | 0.0267              | 0.0009                         | 0.8540                         | 17.2                                 |

<sup>1</sup> Pure, salt free, vacuum dried from the frozen state

<sup>2</sup> Equine paralytic myoglobinuria or azoturia

<sup>3</sup> This corresponds to 19.43 gm myoglobin excreted in 1 day in 900 ml of urine, an abnormal low volume for the horse. In a three day period approximately 60 gm of myoglobin were excreted by this animal.

field where isotopically labelled chromoproteins should supply the answer. e) In the plasma, hemoglobin was slowly oxidized to methemoglobin, while the injected ferri-cytochrome c was rapidly reduced to ferrocytochrome c. The oxidation-reduction potential at pH 7.4 of the plasma is thus physiologically poised between  $\sim +0.13$  and  $+0.27$  volt, the potentials of the respective chromoproteins, and, in this regard, the extracellular environment is not ideal functionally for either hemoglobin or cytochrome c (fig. 1).

possess the necessary enzymic equipment to maintain hemoglobin in an active reduced state. Agreement has been lacking upon the amount of MHb present normally in blood. Our direct spectrophotometric measurements of the oxygen saturation of the arterial blood of man (26) have led us to conclude that the equilibrium  $\text{HbO}_2 \rightleftharpoons \text{Hb} + \text{O}_2$  is usually poised at 0.5 per cent or less of MHb (fig. 1). The same value has been found for normal subjects by Van Slyke *et al* (27). Nonetheless inactive ferrihemoglobin may

be found in large amounts abnormally. The results of the *in vitro* study on dog blood of the reduction of intracellular MHB (fig 2) implicated enzymes concerned with glycolysis, and it was postulated that the DPN and TPN systems could be involved (25). Later Gutman *et al* (28) furnished direct evidence for the rôle of DPN in this interesting and probably physiologically important process. These findings invite a fresh orientation in regard to concepts of methemoglobin production. Methemoglobinemia need not be an expression (perhaps only rarely is) of intoxication by an oxidant of hemoglobin. It may indicate a damage of the enzymic methemoglobin reductive mechanism, or, indeed, a fundamental defect—a 'biochemical lesion'—as has been suggested recently in cases of idiopathic, familial methemoglobinemia (29).

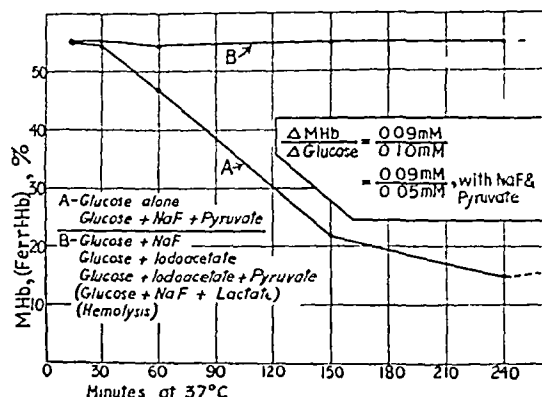


Fig 2 ENZYMIC REVERSION, and its inhibition, of intracellular ferri- or methemoglobin, MHb

*Aspects of the metabolism of cytochrome c* We have used a type of 'depletion technique', partial hepatectomy, in a systematic study of the metabolism of cytochrome *c* in the rat (11, 30-32). This species is particularly suitable for this purpose, since it possesses a discretely multilobed liver, with reproducible ratios of mass of individual lobes to each other (11), and regeneration, following excision of two thirds of the liver, is rapid. In introducing this technique for metabolism studies, it was pointed out (11) that it was a two edged sword, yielding information not only upon the metabolite under investigation but also upon the restoration process *per se*, in itself of fundamental interest.

Our earlier results permitted the following deductions (30, 31): a) The amount of restoration of liver tissue and the quantity of new cytochrome *c* in liver were direct functions of the amount of

tissue removed. b) Most of the liver regeneration and all of the new cytochrome *c* 'production' occurred in the first 4 to 6 days after liver lobectomy. The rate of appearance of new cytochrome *c* was found to be 16, 10.9 and 4.8 per cent per day for restoration periods of 4, 6 and 14 days, respectively. c) The appearance of new cytochrome *c* as well as of ribose nucleic acid, PNA, was relatively independent of dietary protein. The concentrations of each were, significantly, appreciably greater in the regenerating livers from rats on no protein than from those on a high 31 per cent protein diet (30). Apparently partial hepatectomy plus protein starvation offered a maximal deprivation stimulus. The experiments furnished evidence, previously not obtained either by quantitative chemical analysis or *in vivo*, in favor of the view that PNA is involved in cellular protein synthesis in tissue restoration (30). d) A remarkably great liver regeneration (of the order of 170 per cent) was obtained in animals which were shifted, during restoration, from the no protein to the high protein regimen. In these experiments, the results indicated that most of the cytochrome *c* and PNA had been laid down during the period of protein deprivation. When dietary protein was then made available, tissue regeneration 'went to town'.

These studies have led to the conclusion (30) that 'certain cellular components, like cytochrome *c* and PNA, are preferentially produced or deposited in tissues, and are important or essential in growth and proliferative processes, which appear to depend on *intrinsic* (tissue) as well as *extrinsic* (dietary) factors'.

In a separate group of experiments (31) the effects of parenterally administered cytochrome *c* and of anoxia upon liver regeneration and cytochrome *c* in liver were studied. a) Unequivocal evidence was not obtained for the incorporation of injected cytochrome *c* in the regenerating liver, in which a maximal opportunity for such a process should be afforded. On the other hand, in a limited number of experiments increased liver regeneration was found in the cytochrome *c* injected rats. This unexpected finding must be cautiously interpreted, at present, as a nonspecific effect. Anoxia of oxygen deprivation, induced by residence at a simulated high altitude of 15,500 feet (only a moderately high altitude for rats), was without effect either on cytochrome *c* metabolism or on liver regeneration. However, under

these anoxic conditions there was a marked accommodation of the blood oxygen transport system, reflected in large increases of the red blood corpuscles and hemoglobin. Myoglobin was not affected. Thus, although there is doubtless a limit to the effectiveness of this mechanism of adjustment, in these experiments there was a clear separation of a *primary response* to oxygen deficit in the inspired air from any postulated *secondary response*, involving the oxygen utilization mechanism, the cytochrome system, in the

that deeply anesthetized dogs (at low levels of metabolism and, hence, of oxygen requirement) were able to withstand, without any damage to the central nervous system, blood levels of 93 per cent carbonyl hemoglobin (33), whereas unanesthetized animals had 'critical' levels of about 75 per cent HbCO (34).

*Hormonal influence on cytochrome c* The cytochrome *c*-body mass relationship, which has been discussed, and the greater concentration of cytochrome *c* in tissues with higher rates of oxygen

TABLE 3 EFFECT OF THYROIDECTOMY, THIOURACIL AND THYROXINE ON CYTOCHROME *c* (32)

Rats of 200 to 250 gm body weight on high protein diet (30), thiouracil, 50 mg per day, thyroxine subcutaneously, 1 mg every other day

| EXPERIMENT                      | CYTOCHROME <i>c</i>  |                    |                    |                    | CYTOCHROME <i>c</i> IN RESTORED LIVER |             | LIVER RESTORATION | LIVER PNA           | LIVER DNA          |
|---------------------------------|----------------------|--------------------|--------------------|--------------------|---------------------------------------|-------------|-------------------|---------------------|--------------------|
|                                 | Liver                | Kidney             | Heart              | Muscle             | Total                                 | New pigment |                   |                     |                    |
|                                 | $\gamma/\text{gm}^1$ | $\gamma/\text{gm}$ | $\gamma/\text{gm}$ | $\gamma/\text{gm}$ | $\gamma$                              | %           | %                 | mg/gm               | mg/gm              |
| Thyroidectomized                |                      |                    |                    |                    |                                       |             |                   |                     |                    |
| 35 days before liver lobectomy  | 138<br>$\pm 5^3$     |                    |                    |                    |                                       |             |                   | 7.19<br>$\pm 0.10$  | 2.80<br>$\pm 0.05$ |
| 14 days after liver lobectomy   | 181<br>$\pm 5$       | 248<br>$\pm 12$    | 316<br>$\pm 16$    | 57<br>$\pm 3$      | 780                                   | 68.9        | 65.0              | 8.35<br>$\pm 0.11$  | 2.79<br>$\pm 0.06$ |
| Thiouracilized                  |                      |                    |                    |                    |                                       |             |                   |                     |                    |
| 45 days before liver lobectomy  | 145<br>$\pm 3$       |                    |                    |                    |                                       |             |                   | 8.35<br>$\pm 0.21$  | 2.70<br>$\pm 0.03$ |
| 14 days after liver lobectomy   | 165<br>$\pm 3$       | 250<br>$\pm 5$     | 331<br>$\pm 4$     | 55<br>$\pm 3$      | 776                                   | 66.1        | 80.2              | 9.82<br>$\pm 0.40$  | 2.74<br>$\pm 0.05$ |
| Thyroxinized, for 14 to 22 days | 247<br>$\pm 6$       | 422<br>$\pm 8$     | 618<br>$\pm 11$    | 133<br>$\pm 6$     |                                       |             |                   | 11.08<br>$\pm 0.13$ | 2.60<br>$\pm 0.08$ |
| Controls                        |                      |                    |                    |                    |                                       |             |                   |                     |                    |
|                                 | 178<br>$\pm 4$       |                    |                    |                    |                                       |             |                   | 8.50<br>$\pm 0.25$  | 2.46<br>$\pm 0.02$ |
| 14 days after liver lobectomy   | 210<br>$\pm 5$       | 352<br>$\pm 21$    | 447<br>$\pm 16$    | 98<br>$\pm 6$      | 1325                                  | 67.6        | 73.9              | 6.96<br>$\pm 0.24$  | 3.53<br>$\pm 0.04$ |

<sup>1</sup> Wet weight of tissue

<sup>2</sup> 68.4 per cent of liver excised

<sup>3</sup> Values after  $\pm$  are standard errors

tissues (fig. 1). If hemoglobin and the circulation respond adequately to oxygen deficit, there is no need for secondary adjustment in oxygen utilization. Indeed, from a teleological standpoint, the body would lack physiological wisdom if in the face of a diminished oxygen supply, it responded by a greater use of oxygen in the tissues. A temporary advantage might be gained from such a process, but it is a spendthrift's act of desperation, a luxurious meal, which completely empties the diminished stores in the cupboard. Support for this view has been furnished in our finding

consumption (35) directed our attention to an investigation of the influence of hormonal factors on cytochrome *c*. The literature contained a scattering of inconclusive information (36, 37), some of it questionable (37) owing to limitation of results to a single tissue (muscle) and unacceptably low analytical values for cytochrome *c* concentration in normal tissue. To be of significance, a hormonal effect upon cytochrome *c*, a constituent of all aerobic cells, should be demonstrated for all tissues. Table 3 is a summary of mean values obtained by us (32) for the concen-

tration of cytochrome *c* in liver, kidney, heart and skeletal muscle in groups of rats subjected to thyroidectomy, thiouracil thyrotoxicosis (withdrawal of thyroxine by interference with its production) and acute hyperthyroidism induced by injection of thyroxine, in comparison with normal controls. The data appear consistently and conclusively to support the thesis of a relationship of thyroid function to cytochrome *c* in tissues. After both thyroidectomy and thiouracil intoxication there was a striking reduction in total body cytochrome *c*, which was reflected (table 3)

finding of more pronounced liver restoration after thiouracil. This effect was obtained in all the individuals in this group. It may be noted that in the thyroidectomized, thiouracilized and thyroxinized rats the changes in liver PNA are in the same direction as the changes in cytochrome *c*.

It is tempting to draw upon these observations for a concept of how thyroxine regulates the rate of oxygen consumption, since this phenomenon has remained unexplained. The unfolding of the full story must await developments in a study of the influence on cytochrome *c* of other hor-

TABLE 4 LOCALIZATION OF CYTOCHROME *c* IN LIVER CELL (12)

| FRACTION                                   | W D                   |          | CYTOCHROME <i>c</i> |                   |       | DISTRIBUTION OF CYTOCHROME <i>c</i> | DISTRIBUTION OF CELLULAR MASS |      |
|--|-----------------------|----------|---------------------|-------------------|-------|-------------------------------------|-------------------------------|------|
|  | <i>a</i> <sup>1</sup> | <i>b</i> | per gm wet weight   | per gm dry weight | Total |                                     | <i>gm</i> <sup>2</sup>        | %    |
| Liver suspension                           | 18.15                 | 21.10    | 21.3                | 393               | 4155  |                                     | 8.53                          |      |
| Unbroken cells (600 × gravity)             | 7.53                  | 7.78     | 70.0                | 527               | 1895  |                                     | 3.48                          |      |
|  |                       |          |                     | difference        | 2260  | 100                                 | 5.05                          | 100  |
| Nuclear phase (1500 × gravity)             | 11.90                 | 12.75    | 0                   | 0                 | 0     | 0                                   | 0.51                          | 10.1 |
| Mitochondrial phase (9400 × gravity)       | 5.58                  | 5.67     | 263                 | 1470              | 1605  | 71.0                                | 1.07                          | 21.2 |
| Supernatant, microsomal phase <sup>5</sup> | 36.1                  | 19.10    | 3.42                | 123               | 494   | 21.8                                | 2.92                          | 57.9 |

<sup>1</sup> *a* = wet weight to dry weight of original material

<sup>2</sup> *b* = wet weight to dry weight, corrected for added NaCl

<sup>3</sup> Dry weight of tissue

<sup>4</sup> 204 ml of 0.85% NaCl solution containing 34.76 gm of liver parenchyma, recovered from 55.75 gm of whole liver, with an original cytochrome *c* content of 9446 γ. The analytical values are for 180 ml of the liver suspension, containing 30.62 gm of parenchyma. The fibrous portion of liver was 20.99 gm with 3550 γ of cytochrome *c*.

<sup>5</sup> Washings of mitochondrial phase added to supernatant

in statistically significant decreases in cytochrome *c* concentration (as well as content) in all the tissues examined, although the changes were of greater magnitude in skeletal muscle than in other tissues. The administration of thyroxine had the opposite effect, significant increases in cytochrome *c*.

In the thyroidectomized rats, liver regeneration was only slightly less than normal, and the increase in cytochrome *c* concentration, characteristic of regenerating liver (11, 30, 31), was also exhibited in the thyroidectomized and thiouracilized animals (table 3). An unusual result is the

mones, i.e., of the adrenal cortex and possibly of the pituitary. However, our findings permit a tentative postulate. Thyroxine exerts its effect through the agency of cytochrome *c*. As a working hypothesis, this offers a first approximation in a rational chemical explanation of the mechanism of hormonal control of cellular oxygen utilization, in which thyroxine must have a major role. It has the virtue of simplicity in defining the locus of the action of this hormone.

*The cellular localization of cytochrome c.* Several observations invited inquiry as to the state of cytochrome *c* in cells. 1. Myoglobin is readily

extracted with water from ground muscle tissue, which does not release cytochrome *c* to this solvent. The latter pigment remains in the 'press cake', from which (or from the original tissue) it may be isolated, but trichloroacetic (38) or sulfuric acid (14) must be used as extracting agents. These simple facts are interesting. They suggest that myoglobin may be relatively 'free', whereas cytochrome *c* may be 'bound' in the cellular structure.

2. The activity (in terms of oxygen uptake) of cytochrome *c* in intact tissues (35) is of an appreciably higher order of magnitude than that obtained by the addition of the best cytochrome *c* preparations to tissue homogenates (39)—a fact insufficiently recognized.

3. In the regenerating liver, as has been pointed out, parallel changes in cytochrome *c* and PNA were found (30). By means of the differential centrifugation technique developed in Claude's laboratory, PNA had been shown (40) to be a constituent of cytoplasm, and cytochrome oxidase was demonstrated to be localized exclusively in the cytoplasmic large particle aggregates, the mitochondria (41). This suggested the possibility that cytochrome *c* might be similarly localized (30). The idea is now beyond the speculation stage. While we were engaged in testing it by applying Claude's methods, Schneider, Claude and Hogeboom (42) proved the point in their very recent contribution.

Our tally sheet for the internal cellular distribution of cytochrome *c* is supplied in table 4. The results essentially confirm those of Schneider

*et al.*, although we find an appreciably higher localization in the mitochondrial phase, 71 per cent in the large aggregate fraction after washing with 0.85 per cent NaCl as against their 40 per cent (42). It also appears probable that at least some (if not most) of the microsomal phase may be an artifact, representing the breakdown of the large aggregates. If that is the case, nearly all of the cytochrome *c*, like cytochrome oxidase, may have been *in vivo* localized in the mitochondria. This would be consonant with our finding (35) that the determination of cytochrome *c* can be used as an index of the activity of cytochrome oxidase in tissues. Aside from other considerations, such a localization increases manifold the effective reacting concentrations of cytochrome *c*, the substrate, and cytochrome oxidase, the enzyme (fig. 1).

Derived from an application of the newer cytochemical techniques, particularly that of Claude, is a fascinating picture of the mitochondria, floating batteries carrying cargoes of enzymic charges on a sea of cytoplasm. Enticing vistas are opened. The avenues of investigation of cytochrome *c* converge—its relationship to body mass, the proportionality of its cellular concentration to the oxygen consumption of the tissue, its intracellular orientation with its oxidase and the suggestive nature of its hormonal control.

A large part of our work on the metabolism of cytochrome *c* has been done under contract between the Office of Naval Research and the University of Pennsylvania.

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# METABOLISM OF IRON

PAUL F. HAHN

*From Meharry Medical College, Nashville, Tennessee*

Our knowledge of the metabolism of iron has been greatly increased during the past decade largely due to the advent of the radioactive isotopes of this metal. Iron is present in the body in a wide variety of forms of combination. By purely chemical methods alone it had proved practically impossible to trace the course taken by recently ingested iron. The presence of larger amounts of it in various forms in the tissues under examination masked the presence of the relatively minute amount added as a result of feeding. As in the case of other metabolites, tracer studies with isotopes led to the solution of many of these problems. Iron plays a vital part in the metabolic activity of every mammalian body cell. Cytochrome, catalase and muscle hemoglobin iron are husbanded by the body regardless of the stresses imposed by serious deficiency disease. Some other iron fractions such as plasma iron, on the other hand, are extremely labile. The bulk of the body iron exists in the red blood cell hemoglobin and much information has been and promises to be learned about the formation and breakdown of this compound. Application of the newer methods of research in this field requires a high degree of collaborative effort on the parts of the physicist, chemist, biologist and clinician. Such collaboration probably represents the pattern of much future medical research.

In no sense shall I attempt to review here what has been done in the whole field of iron metabolism. Rather I shall attempt to bring out the significance of a few of the more recent developments and to point out a few of the more glaring deficiencies in our present state of knowledge.

One of the first contributions made with the use of the  $^{59}\text{Fe}$  isotope was related to the unique ability of the body to accept or reject iron following ingestion, depending on the need for the element (11). Selective absorption had been suspected as a result of the findings of Welch, Wakefield and Adams (33) in which no excretion of iron was demonstrated in a patient with an ileostomy stoma and an isolated colon. McCance and Widdowson (23) later showed that even following the injection of considerable amounts of

iron by vein, there was a negligible amount of excretion. On the basis of these balance studies they had the courage to suggest that selective absorption prevented an accumulation of undesirable amounts of this element in the body. With the isotope it was possible to recognize the presence of recently administered iron in plasma and later in the red cell hemoglobin and to show that in iron-deficiency anemia the absorption was increased many times over the normal absorption. This finding has been corroborated many times since in our and other laboratories. That anemia *per se* did not influence the uptake of the metal was seen from the fact that normal dogs bled acutely about 60 per cent of their estimated blood volumes when fed tagged iron absorbed no more than when in the normal state. However, if a week or so were allowed to elapse following the hemorrhage, the uptake of tagged iron was several times what it had been (10, 17). This finding led us to postulate an acceptor mechanism in the gastro-intestinal mucosa which normally was saturated with iron and, therefore, allowed a minimal amount of passage from the lumen to the plasma.<sup>14</sup> The interesting work which was currently being done by Granick and Michaels (6, 7, 24) on the properties of ferritin pointed to the possibility of that compound being the intermediary responsible for this unusual phenomenon. Accordingly, a joint study between the Rochester and Rockefeller Institute laboratories was set up and it was shown that inorganic iron could be converted readily to ferritin iron in the liver. Further, it was demonstrated that hemoglobin iron upon the destruction of red cells by acetylphenylhydrazine was in part, at least, converted to ferritin in the liver and spleen. Thus the storage function of ferritin was established (14). Granick continued the work (8, 9) and was able to demonstrate larger amounts of ferritin in the duodenum than in any other part of the G-I tract. Furthermore, he discovered that there was an increase in the amount of ferritin in the intestinal tract when animals were fed iron repeatedly. This very interesting finding suggests an ability of the body to acclimate itself to iron feeding and deserves

considerable study since such a mechanism might be an important factor in the efficiency of therapeutic measures. Unfortunately, little has been done to date in studying the comparative uptakes of single or multiple doses of iron. Such studies could only be carried out successfully with the isotope method.

*Distribution of iron* No studies of the true distribution of iron in the human body have been carried out up to this time. Chemical determinations have been obscured by the presence of blood in tissues. However, using the vivoperfusion technique values have been obtained for dogs by Bogiard and Whipple (2) and Hahn and Whipple (19) and for rats by Auston, Rabinowitch and Greenberg (1). For the distribution in dogs see table 1.

TABLE 1

|   | % TOTAL<br>BODY IRON |
|---|----------------------|
| Blood hemoglobin iron   | 57                   |
| Muscle hemoglobin iron  | 7                    |
| Total hemoglobin iron   | 64                   |
| Parenchyma iron (catalse, cytochrome,<br>peroxidase, etc.)  | 16                   |
| Available visceral storage (ferritin,<br>hemosiderin, etc., in liver, spleen,<br>and bone marrow) | 15                   |
| Available iron, other tissues (estimated)   | 5                    |
| Total iron  | 100                  |

These values were arrived at by analysis of dogs of about 20 kg weight with a circulating blood volume of about 1500 ml and a bulk of striated muscle estimated at 6.5 kg. Since the muscle hemoglobin and parenchyma iron are unaffected by hemorrhage or dietary depletion, the available stores of iron are such as to allow a 30 per cent replacement of the blood hemoglobin in time of need.

*Efficiency of iron absorption* Fed at anything other than ridiculously low levels the absorption of iron is notoriously inefficient. When chronically anemic human subjects or animals are fed doses of tagged iron at 0.2 to 1.0 mg levels there may be as much as 50 to 90 per cent uptake (18). When levels such as might occur in the normal dietary are fed, i.e., 50 mg, the growing child was found to absorb only about 12 per cent (4). In the latter stages of pregnancy when the need

is augmented, 50 to 180 mg doses of the tagged iron administered result in the uptake of only about 35 per cent (13).

Too little attention has been paid by clinicians to the work of Whipple and Robschert-Robbins (34) in this matter. Many years ago they showed that in their standardized anemic dogs iron fed at a daily level of 40 mg over a two-week period was absorbed and utilized to the extent of 35 per cent. When the dosage level was increased to 100 mg per day the percentage uptake was only 5 to 6 per cent. Thus, an increase in dosage level by a factor of ten resulted in less than double the absolute uptake. These findings are in agreement with those obtained using single feedings of tagged iron. Since the commonly used therapeutic dose is 325 mg one may expect only about 16 mg of actual iron absorption. This is the equivalent of about 5 gm of hemoglobin. The normal adult male has about 750 gm of blood hemoglobin in circulation and this may frequently be reduced to one third or less by acute or chronic hemorrhage. Thus, it becomes apparent that iron must be taken assiduously for many weeks in order to make up losses due to massive acute hemorrhage.

At present there is nothing known to enhance the absorption of iron. On the other hand, many reports indicate that phosphates, phytates and other dietary ingredients reduce the absorption. It would, therefore, seem advisable to administer iron between meals when given either prophylactically or therapeutically.

*Valence state and iron uptake* Clinical experience had shown that iron in the ferrous form seemed to be a more suitable means of providing the patient with iron than the corresponding ferric salts. That the latter salts are poorly tolerated was well known and probably influenced the physician considerably in his thinking and practice. However, this presumption was probably largely based on empiricism. Whipple and Robschert-Robbins (34) had found in their multiple feeding experiments in dogs that there was little if any difference in uptake whether ferrous, ferric or reduced iron was administered. In single feeding experiments in both dogs and human subjects we found by using the tagged iron method that ferrous salts were many times more efficiently absorbed (15, 16). Moore and his colleagues (27), however, found ferrous iron more readily absorbed in human patients but noted no difference in the uptake in dogs. The latter group have suggested that a species difference may exist,

but we feel that our data shows conclusively that the disparity obtains in both species

From a more practical aspect it has been possible to demonstrate that the need for iron determines its uptake. Feeding a standard test dose of tagged iron to 176 school children in the 7 to 10 year age group, we found that the uptake was closely correlated (4) with Heath and Patek's (20) estimated yearly increases in body iron during growth

More recently in a Cooperative Study of Infant and Maternal Nutrition conducted in Nashville during the past two years, nearly 1000 pregnant women were fed tagged iron. It was found that there was from 50 to 100 per cent greater absorption of iron in the last quarter of pregnancy than in the first quarter (13). This is an excellent reflection of the fetal growth demand. Further, it indicates that the logical time for prophylactic

TABLE 2 UPTAKE OF RADIOACTIVE IRON AT VARIOUS STAGES OF GESTATION IN 329 PREGNANT WOMEN

| NO PTS<br>IN GROUP | UPTAKE AT WEEKS OF GESTATIONAL PERIOD |       |       |       |
|--------------------|---------------------------------------|-------|-------|-------|
|                    | 0-10                                  | 11-20 | 21-30 | 31-40 |
| 22                 | 18.2                                  |       |       |       |
| 122                |                                       | 21.4  |       |       |
| 108                |                                       |       | 31.5  |       |
| 77                 |                                       |       |       | 38.5  |
| 329                |                                       |       |       |       |

and replacement iron medication is in the last half of pregnancy, at which time the iron will be most efficiently taken up. Prescribing iron early in pregnancy should reasonably be avoided since the saving from lack of catamenial blood loss will more than repay the early fetal needs. Furthermore, a woman's good intentions to take her thrice daily pills will have long since been forgotten when the supplement would do the most good.

Transport of iron That the blood plasma is the vehicle of iron transport is now generally agreed. On being absorbed from the gastrointestinal tract it is felt that the iron is oxidized to the ferric form and combined firmly with plasma globulin (21, 31). This transport function has been studied extensively by Moore and his group (25, 26), Waldenstrom (32), Laurell (22) and by the use of the radioactive isotope by Hahn, Bale, Lawrence and Whipple (11) and Yoshikawa, Hahn

and Bale (35). Depletion of the labile stores has been shown to be reflected in a lowered serum iron level (21, 22, 26). Normal values may range from 80 to 200  $\mu\text{g}$  per 100 ml. In iron deficiency anemia or pernicious anemia in remission, the serum iron may be markedly lowered, whereas in pernicious anemia in relapse, it may be somewhat elevated.

Many attempts have been made to quantitate the amount of iron absorption from the intestinal tract by means of the rise in plasma iron levels. However, it should be kept in mind that at least two processes are occurring simultaneously, namely, passage from the lumen of the gut to the bloodstream and removal from the latter by the storage depots and the hemopoietic system. That these processes may have different rates under various circumstances seems altogether likely and, therefore, such quantitation attempts are probably highly unreliable.

At this writing, no clear-cut demonstration of the transport of iron from the storage depots to the bone-marrow has been made.

The importance of the plasma iron fraction should not be underestimated merely because of the relatively small amounts appearing in the circulation at any one time. The alterations in level mentioned above are of great clinical significance. The depression of plasma iron levels in inflammatory and infectious processes is under study by Cartwright, Wintrobe and their collaborators (3) using the radioactive isotope and should add considerably to our knowledge of the metabolism of this element in disease.

Inflammation and iron absorption and utilization In cachexia, many inflammatory disorders and infectious disease, there is a high incidence of accompanying anemia. This condition, though generally recognized, is not at all understood. Some think that toxic factors are at play with a resultant depression of the bone marrow activity. It is well known on an empirical basis that iron therapy is of no value while the disease process is rampant.

Robscheit-Robbins and Whipple demonstrated in a dog with chronic endometritis a markedly decreased rate of utilization of iron (30). Following hysterectomy there was a resumption of the usual hemoglobin production.

Using tagged iron and the turpentine sterile abscess as a means of inducing a controllable inflammatory process, we were able to show that an abscess induced in the axillary region was able

to depress iron absorption considerably (12) The results of this set of experiments can be seen in table 3 Some might argue that there had been absorption without subsequent utilization of the iron Therefore, in the case of one animal, the

TABLL 3 IRON ABSORPTION BY DOGS WITH SUBCUTANEOUS TURPENTINE ABSCESS'S

| DOG NO | HEMA-<br>TOCRIT | FORM ID<br>MINISTERED | DOSE | UP<br>TAKE | EXPERIMENT<br>TYPE                           |
|--------|-----------------|-----------------------|------|------------|--|
|        | %               |                       | mg   | %          |  |
| 40-149 | 13 4            | FeCl <sub>3</sub>     | 9    | 41         | Control                                      |
| "      | 23 0            | "                     | 10   | 4 1        | Turpen-<br>tine 1<br>day pre-<br>viously     |
| "      | 18 3            | "                     | 10   | 15 1       | Control                                      |
| 41-164 | 26 9            | Fe Am Cit             | 38   | 4 1        | Turpen-<br>tine 2<br>days<br>previ-<br>ously |
| "      | 28 6            | "                     | 34   | 17 6       | Control                                      |
| 37-196 | 22 4            | "                     | 38   | 5 3        | Turpen-<br>tine 2<br>days<br>previ-<br>ously |
| "      | 23 4            | "                     | 34   | 6 9        | Control                                      |

<sup>1</sup> This animal had been splenectomised and, therefore, was subject to Bartonella infection That this infection was responsible for lowered uptake at this time as suspected by clinical condition seemed indicated by reaction to treatment with Mapharsen

Balance sheet for first feeding of dog 37 196  
10 days after feeding, Hct 35 2%,

Estimated total circulation radio iron  
1720 cpm  
2 weeks later, Hct 28 7%,  
Estimated total circulation radio iron  
635 cpm

Radioactivity lost to circulation 1085 cpm  
Radioactivity found in 1900 ml blood re-  
moved in two-week interval 4045 cpm  
This balance shows that with the turpentine ab-  
scess there was no additional absorbed and stored  
iron available for use under the stimulation due  
to blood loss

actual amounts of total radioactivity removed by  
repeated bleeding was determined and was found  
to agree very favorably with the difference in  
circulating amounts of tagged iron before and  
after bleeding This indicated that there had been  
no storage of the fed isotopic iron

Recently Gibson and Finch (5) have been  
studying the effect of infection on iron utilization  
of tagged iron administered intravenously in hu-  
man subjects "The results obtained showed that  
the utilization of iron for hemoglobin synthesis  
was impaired to a degree roughly proportionate  
to the severity of the infection as judged by  
clinical evidence"

Thus we see that inflammation and infection  
interfere both with the absorption and utilization  
of iron Much remains to be done in elucidating  
the mechanisms of these reactions

*Pathway of iron from red cell hemoglobin break-  
down* It seems reasonable to allow a little specu-  
lation to enter the scene at this point regarding  
the path of breakdown of hemoglobin From the  
work of Lemberg, Whipple and Barkan and then  
associates, it would be inferred that the picture  
would be somewhat as follows "Upon completion  
of its four-month life cycle the red cell having  
deteriorated in some unknown manner would be  
phagocytosed by the cells of the lymphoid-macro-  
phage system The released hemoglobin would  
then undergo splitting at the alpha methene  
linkages of the porphyrin part of the molecule  
giving rise to verdo-hemoglobin The iron would  
then be split off and stored in the Kupfer cells  
and macrophages for future use The globin would  
then become dissociated from the porphyrin de-  
rivative and enter the protein metabolic pool  
The bilirubin would be quantitatively secreted  
by the biliary tract" Another possible pathway  
presents itself, suggested by some rather provoca-  
tive data accumulated over the years in studying  
the iron isotope Under conditions of red cell  
destruction due to hydrazine derivatives it ap-  
peared that iron from recently destroyed red cells  
was utilized preferentially, regardless of the  
amount of readily available storage iron that  
might be present in the liver, spleen and bone  
marrow Also, as has been pointed out, it should  
be kept in mind that under these conditions  
tagged red cell iron has been shown to be de-  
posited in the form of ferritin This latter material  
is a non-protein compound containing up to  
23 per cent of its weight of the metal and having  
a molecular weight of 460,000 (9) Under condi-  
tions of acute or chronic blood destruction path-  
ologists have for many years recognized iron in  
the form of hemosiderin by the Prussian blue  
staining reaction Since hemosiderin and ferritin  
are laid down under the same conditions in the  
same cells, it is only reasonable to inspect the

possibility of their being either identical or closely related. Simplification of our view of the path taken during the destruction of hemoglobin could be accomplished if it were not for a few observations. Globin has a measured molecular weight of about 68,000. This does not preclude the possibility, however, that apoferritin might be an aggregate of globin. Much confusion stemmed from the arbitrary classification of proteins through the salting-out technique which is being clarified by modern electrophoretic studies. In this connection it should be well to recall the similarity between serum albumen and globin as shown by Pederson and Wildenstrom (28).

Tentatively, and at the risk of severe criticism from those much better versed in the chemistry and structure of proteins, I should like to suggest a slightly different pathway of hemoglobin disintegration than that outlined above. Upon phagocytosis of the red blood cell let us suppose that following splitting at the methene linkages the porphyrin entity of the molecule is immediately divorced from the non-globin residue, becoming attached to serum albumen or not, as the case may be, and undergoing secretion by the hepatic-biliary system. The non-globin complex would then be deposited as a precursor of hemosiderin and/or ferritin under some conditions, or would be transported to the bone marrow for immediate incorporation into the megaloblast, reticulocyte or normoblast, depending on which of these forms of the red cell are actually the site of hemoglobin formation. Such an hypothesis should lend itself readily to test using  $N^{15}$  as a tag in the globin fraction of the hemoglobin molecule.

*Problem of iron deficiency anemia.* Etiologically, iron deficiency anemia may be classified as follows: a) hemorrhagic, acute or chronic, b) nutritional, c) parasitic, with or without nutritional complications, d) gestational, with or without hemorrhagic or nutritional complications, e) other obscure or unusual causes.

a) It has been stated repeatedly elsewhere that microcytic, hypochromic anemia does not occur in the normal male adult unless there has been blood loss. The common causes of acute hemorrhage include gastric and duodenal ulcers, ruptured esophageal varices, uterine fibromata, tumors of the gastrointestinal tract, premature separation of the placenta, etc. These conditions nearly all also contribute largely to the causes of chronic hemorrhage, to which should be added hemorrhoids,

menorrhagia, metrorrhagia, hemorrhagic diathesis due to leukemia, etc. There is still some disagreement as to the advisability of transfusion in acute massive hemorrhage as an immediate measure for fear of inducing further hemorrhage. Each case presents an individual problem. However, there is no doubt that iron therapy is indicated for both massive acute and chronic hemorrhage. Due to the inefficient absorption of iron in oral therapy the element in suitable form must be administered frequently and continuously for many weeks in order to care for replacement and storage needs. There being no suitable preparation of iron for parenteral use at the present the only means by which large quantities of iron may be administered in a short period is by infusion of whole blood or suspended red cells. When there is a chronic low grade blood loss it is a simple matter to maintain the patient in equilibrium as long as replacement therapy is faithfully adhered to. For such treatment it is recommended that small doses of 30 to 60 mg of iron as the ferrous salt be administered at frequent intervals, preferably between meals, over prolonged periods of many weeks.

b) Nutritional iron deficiency anemia is a relatively rare condition in the United States. It is probable that only 5 to 10 mg of absorbable iron are needed to take care of the growth requirements of children and the catamenial losses in women. The requirement for men is probably only a fraction of this. Since our average dietary contains well over this amount, we see very little uncomplicated anemia due to lack of intake of the element. Where it exists it may be a result of local peculiarities of diet or in individual instances, a result of food fadism. Nutritional anemia in northern Brazil, India and China constitutes a serious problem on the other hand, especially when the deficient dietary is complicated by pregnancy or parasitism.

c) Parasitism may play an important role in iron deficiency anemia as perhaps best exemplified by the indigent population of Brazil. In the northern part of the latter country where the worm burden due to ankylostomiasis may reach 400 or more per person, the incidence of hookworm anemia is very high. Here the dietary consists largely of rice and black beans. On the other hand, although the worm burden in the same class of population in the southern part of Brazil may be equally as high, the incidence of anemia is low. Presumably, this is because of the high beef content of the dietary supplying sufficient replacement of red blood cell hemoglobin iron.

d) Gestational anemia may be due to multiple frequently occurring pregnancies, especially where there has been no prophylactic iron taken late in pregnancy or where the dietary is inadequate to take care of the extra demands. Other common

causes are excessive hemorrhage at parturition or post-partum

e) Chronic infection or some other process which interfered with normal iron uptake may, in the presence of unusual demands for iron such as occur during growth or pregnancy, result in an iron deficiency condition. We have seen an instance where pernicious anemia in relapse in a growing child (a condition marked by lowered iron absorption) resulted in a superimposed iron deficiency

anemia which became apparent on institution of specific therapy with liver extract

The replacement requirements for iron therapy can be defined simply. The chief difficulty is in estimating the true blood loss or deficiency. When the latter can be estimated one may apply the value of 0.5 mg. of iron needed to be absorbed for each ml. of blood of normal hematocrit value lost.

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# A SYSTEMATIC TREATMENT OF COORDINATION COMPLEXES OF METALLOPORPHYRINS AND NITROGENOUS BASES

W MANSFIELD CLARK,<sup>1</sup> T H DAVIES, C C PORTER, J F TAYLOR, JOSEPH SHACK, C S  
VESTLING AND J R WEISIGER

*From the School of Medicine, The Johns Hopkins University, Baltimore, Maryland*

Systems containing metalloporphyrins and coordinating substances may exhibit complex equilibrium states involving exchanges of electrons, protons and coordinating substances and the making and breaking of dimeric units

Because of the complexities it is impracticable to include in this review more than a sketch of the sort of data assembled by the authors, or published by Conant *et al*, Hogness *et al*, Barron and others (1) Nor is it practicable to reproduce here the numerous diagrams which, with the exception of those illustrating new data, have been published

Measurements of diffusion coefficients, and some comparative tests by means of the ultracentrifuge confirm earlier, scattered evidence that metalloporphyrins in aqueous solution form large micellae Some of the following data indicate that within these micellae are dimeric units The bondings in the larger aggregates and even in the dimers appear to be too weak to be reflected in those energy changes which are involved in some of the measurements of equilibrium states, but the bonding of the dimeric units is definitely reflected in some of the measurements This poses an interesting problem in methodology

The resulting complexity has made it advisable to preserve, so far as practicable, objective mathematical methods of analyzing data The methods of Reed and Berkson (2) have been used in most cases The limitations set by the precision of the data have been noted in previous publications

Porphyrins, such as protoporphyrin IX and the corresponding meso- and hematoporphyrins and coproporphyrin I, coordinate with any one of several metals to form a class of substances which we call metalloporphyrins The iron, cobalt and manganese complexes are subject to oxidation-reduction We have determined the electrode potentials of a considerable variety of

these systems In some instances the more reliable potentials were obtained by use of a mediator, that is, a reversible oxidation-reduction system which affects the electrode the more definitely, keeps in equilibrium with the system under study and is present in proportions so small as not seriously to affect the state of oxidation-reduction of the principal system Correction of the data for the interaction can be made easily when the proportions of the two systems and the characteristics of the mediator system are known For example, previous investigators have had difficulty in obtaining steady electrode potentials with the non protoporphyrin system By use of indigosulfonates as mediators Shack and Clark obtained reliable data

Whereas a ferri porphyrin contains hydroxyl ion a ferro porphyrin does not This is evidenced by the fact that such a system at fixed degree of reduction shows a change of potential with change of pH defined by  $\frac{\Delta E}{\Delta \text{pH}} = -0.06$  at 30°C The cobalt and manganese porphyrins show no such change

The shift of potential with pH is not attributable to a great change in the ionization constants of the carboxyl groups of proto-, meso- or coproporphyrin This is a reasonable assumption checked by several facts In spite of the low solubility in neutral and acid solution ferri coproporphyrin I was found by Porter to give a titration curve indicating that all the carboxyl groups are ionized in the region of pH to be considered in the following cases Secondly, etioporphyrin, which contains no carboxyl groups, showed with pyridine the relation  $\frac{\Delta E}{\Delta \text{pH}} = -0.06$

If we assume that iron has the coordination number 6, and that four positions are occupied by the four pyrrolic nitrogens of the porphyrin ring, there remain two positions to be filled We consider it a stitistic and not experimentally proven that in aqueous solution water molecules can fill

<sup>1</sup> Paper presented by W Mansfield Clark

these positions. If so, the finding of evidence for coordinated  $\text{OH}^-$  can be explained by loss of a proton from one of the coordinated water molecules. This should be replaced at relatively low pH. Measurements of this ionization constant are difficult because of the low solubility of any one of the metalloporphyrins, but we have spectrophotometric measurements indicating that the half-transformation points are at about pH 7.4 to 7.6.

A metalloporphyrin will coordinate with any one of a large number of nitrogenous bases such as pyridine, nicotine, pilocarpine, histidine and with cyanide ions.

If compared under comparable conditions, such compounds show striking differences in the association constants. For example, the concentration of pyridine required for half-conversion of ferri-mesoporphyrin to the pyridine complex may be as high as 0.3 molar whereas under comparable conditions pilocarpine will half-saturate the metalloporphyrin at 0.3 millimolar concentration. On comparing substituted pyridines Weisiger finds effects reflecting their ionization constants and also evidence of steric hindrance.

In several cases the evidence points to stepwise additions to a reduced metalloporphyrin. Although there are theoretical reasons for assuming step-wise additions to oxidized metalloporphyrin the data can be treated mathematically as if the ratio of equilibrium constants approaches zero, that is, no intermediate forms to a surely detectable extent.

In the more usual case the coordination with the reduced metalloporphyrin is the stronger. Hence the potential of the system at a given per cent reduction becomes more positive as concentration of the coordinating substance increases. This shift of potential is a reflection of the difference between the free energies of association.

Analyses of spectrophotometric data on the association between a ferri porphyrin (proto-, meso-, or copro-) and either pyridine, nicotine or pilocarpine in alkaline solution indicates that two molecules add per unit of metalloporphyrin. Apparently neither of these replaces  $\text{OH}^-$ , which would seem to be confirmed by the fact that the

relation of electrode potential to pH is  $\frac{-\Delta E}{\Delta \text{pH}} =$

0.06. In neutral solution, however, the data suggest that with the elimination of  $\text{OH}^-$  from a ferri-porphyrin four molecules of pyridine add to the dimeric metalloporphyrin, split the dimer and form monomeric dipyrindine ferri porphyrin.

In all cases it appears that the reduced ferro porphyrin is also dimeric but adds pyridine, perhaps stepwise to form monomeric complexes. These do not vary with pH.

Consequently reduction of the dimeric dipyrindine ferri porphyrin should involve two electrons per unit and should be accompanied by the elimination of  $\text{OH}^-$  and the splitting of a dimer to a monomer. The available evidence supports this.

As was shown by Hogness *et al.*, the spectrophotometric data on the coordination of cyanide ion with ferriprotoporphyrin can be accounted for on the assumption that four cyanide ions add to this dimeric metalloporphyrin, eliminate  $\text{OH}^-$  and split the dimer to form monomeric dicyanide ferriprotoporphyrin. We have extended this study to show that HCN does not combine with a ferriporphyrin but only cyanide ions, and that the association is a function of both the ionization constant of hydrocyanic acid and the ionization constant of the hydroxyl of ferriprotoporphyrin.

Any one of these systems when saturated with  $\text{CN}^-$  gives an electrode potential invariant with pH. The curve relating potential to pH at fixed degree of reduction crosses the corresponding curve for the metalloporphyrin and indicates that when released from the necessity of competing against  $\text{OH}^-$  in the oxidant,  $\text{CN}^-$  combines more tightly with ferriprotoporphyrin than with ferroprotoporphyrin. The reverse is true of alkaline solutions. This suggests certain analogies with the effect of cyanide on enzyme systems. The analogy may not be good as will appear in the following remarks.

If to an aliquot of a solution of an iron porphyrin there is added  $\text{CN}^-$  sufficient to cause some but not an extensive formation of a complex and to another aliquot there is added pyridine or pilocarpine sufficient to cause a little but not an extensive formation of a complex, an extensive color change will take place on mixing these solutions. The extent of the formation of what appears to be a new complex is a complicated function of the concentrations of the reacting substances and of pH.

One of the more striking facts is the extremely low concentrations of the coordinating substances required to form the complex. This rules out the formation of a mixture of those complexes which are formed on the one hand with cyanide alone and, on the other, with pyridine or pilocarpine alone. We have accounted quantitatively for this phenomenon by assuming the formation



of a monomeric, monocyanide, monopyridine ferri protoporphyrin

A first approach to an account of this fact is found on considering the competition between coordinating substances and hydroxyl ion for place in the coordination complex. Cyanide ion can compete successfully, replacing  $\text{OH}^-$ , pyridine cannot. But on lowering pH, so that pyridine does not have to compete with  $\text{OH}^-$ , we find pyridine behaving as does cyanide. Remove the competition of  $\text{OH}^-$  by means of  $\text{CN}^-$  and pyridine enters on about the same footing. This description is, of course, a bit crude and is resorted to only to give the over-all picture.

No account of a few of the conclusions which have been confirmed would be fair without remarking that each fresh attack reveals some hitherto unsuspected complexity. For example, some earlier work on the complexes formed with pilocarpine was repeated after a study had been

made of the kinetics of hydrolysis of the lactone. The author thought he had found a striking lowering of the affinity of pilocarpine for ferri protoporphyrin due to the opening of the lactone ring. A repetition with unhydrolyzed pilocarpine under conditions such that hydrolysis could not have been of significant rapidity gave essentially the same association curve. What is remarkable is that, unlike all previous experience, the curve analyzed as if but one pilocarpine added. The only difference between these cases and those previously studied which has been detected to date is that in the latter cases the solution of ferric protoporphyrin in a borate buffer had 'seasoned' by standing for several days.

We already have published a note on the spectral changes occurring in a solution of 'heme' (ferri protoporphyrin) on standing. The spectral changes suggest alteration of degree of aggregation accompanied by slower decomposition.

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# RELATION OF PHYSIOLOGICAL FUNCTION AND MOLECULAR STRUCTURE IN HEMOGLOBIN

JEFFRIES WYMAN, Jr

*From the Biological Laboratories, Harvard University, Cambridge, Massachusetts*

The most important physiological functions of hemoglobin in the vertebrate body are probably to provide for the transport of oxygen, to participate in the transport of carbon dioxide and to act as a buffer in maintaining constancy of the pH of the internal environment. The first of these is due primarily to the presence of four hemes in the molecule and then interaction, the third to the characteristics of the globin and the second largely to the character of the heme globin bonding, which involves the existence of certain oxygen-linked acid groups. These groups operate as a very simple and beautiful physicochemical mechanism in accordance with the principles of acid-base equilibrium. All in all, hemoglobin certainly represents one of the most striking examples, if indeed it is not *the* most striking example, known to biochemistry of the relation of structure to function at a molecular level. It is the purpose of this paper to develop this point of view a little further.

We shall begin, by way of general background, with a brief summary of existing knowledge with regard to the physical and chemical characteristics of the vertebrate hemoglobins. In all of these there are four protohemes in each molecule, and the molecular weight is close to 67,000, corresponding to an iron content of approximately 0.35 per cent. In the case of horse hemoglobin, which is probably the most completely characterized of the hemoglobins, it may be deduced from physicochemical studies that the molecule in solution may be approximated either by an oblate ellipsoid with an axial ratio of 3.1 or a prolate ellipsoid of axial ratio of 1.3, and contains about 30 per cent water of hydration (20). As between these alternatives, a clear decision is provided by the x-ray studies of Perutz and his associates (1), whose conclusions go much farther. They have inferred that in the dry crystal the molecules of horse ferrihemoglobin are right circular cylinders with convex ends, 31 Å high and 57 Å in diameter, consisting of two identical halves and having a two-fold axis of symmetry. They contain four equally spaced layers of polypeptide chains. The four hemes occur in pairs on the surface of the molecule, with their planes parallel to one another and to the plane defined by

the cylinder axis and the axis of symmetry. On the basis of osmotic and centrifugal studies it is known that at high dilution or in strong urea solutions, the molecule splits into two equal parts (11, 14).

As regards the amino acid composition, the most striking feature is the almost uniquely high figure for histidine (33 residues per molecule, 16). Recent studies of Singer and Porter<sup>1</sup> give information on the number of free  $\alpha$  amino groups in several mammalian hemoglobins. In the case of the horse there appear to be six of them, all belonging to valine, in the case of cow, sheep and goat there are four, two belonging to valine and two to methionine. This illustrates the fact that there are significant differences of composition as well as of physicochemical properties between hemoglobins of different species.

With this preface, let us now consider the buffering action of hemoglobin. The total number of titratable groups in horse hemoglobin is estimated as about 178, but most of these are only active at rather extreme reactions. The groups which may be expected to be active in the physiological range are the 33 imino groups of the histidine residues and the six  $\alpha$  amino groups of valine revealed by Porter and Sanger. Owing to the proximity of an imide linkage the  $pK$  of these latter groups might be lowered towards 7. In histidine itself the  $pK$  of the imino group at 25° is 6, and in the histidine peptides which have been studied it is somewhat less (5.6–5.8) (2, p. S5). Between pH 6 and 8 the buffering power of horse hemoglobin remains fairly constant at the high value of approximately 2.9 equivalents per heme per pH unit, which is the value characteristic of the middle of this range and is essentially the same for hemoglobin as for oxyhemoglobin (6). This accounts for the remarkably high pH stability of the blood under physiological conditions. That the acid groups involved are in fact primarily imino groups of histidine records well with the effect of temperature on the titri-

<sup>1</sup> Personal communication from Professor A. C. Chibnall.

tion curve, from which an apparent heat of dissociation of approximately 6200 calories, involving about 31 groups active between pH 5.5 and 8.5, may be deduced (17). This is a value characteristic of the imidazole group of histidine. These considerations offer the first instance of the important physiological role played by this amino acid, namely its action as a buffer.

We turn now to the question of oxygen transport. The bulk of the evidence, including that from x-ray studies, heats of oxygenation and Bohr effect, is in favor of the view that the four hemes are all alike in their reactivity with oxygen, and that each is linked with an essentially identical configuration in the globin. They are not, however, independent of one another, but are subject to stabilizing interactions such that the introduction of one oxygen molecule into the protein facilitates the introduction of a second as by a kind of decoying mechanism. This follows unequivocally from the value of  $n = 2.5 - 3.0$  in the approximate Hill equation. A particularly simple and ingenious model of how that might occur has been given by Pauling and is very instructive (8). It is now clear, however, that it cannot be regarded as meeting the most exact experimental facts. Instead, it seems highly probable, on the basis of a more recent analysis (21) involving experiments on the oxygen equilibrium of hemoglobin split in strong urea solutions as well as experiments on oxidation-reduction voltages of both native and split hemoglobin, that the four hemes occur in two identical pairs with strong interactions between members of the same pair and weaker interactions between members of different pairs. The resulting effects on the steepness and sigmoid character of the oxygen equilibrium curve, which are of obvious physiological significance, are a particularly good example of the relation of structure and function in this remarkable molecule. As regards the origin of the stabilizing energy between the hemes, it may be inferred from the effect of splitting the molecule, which causes an increase in oxygen affinity, that in the case of hemes belonging to different pairs it involves primarily the unoxygenated forms. Without dwelling further on these matters, however, let us turn now to those aspects of the oxygen equilibrium which relate directly to the rôle of the oxygen-linked acid groups, groups which, as we shall see, are probably to be identified with histidine.

A knowledge of the oxygen Bohr effect, by which is to be understood the effect of pH on the oxygen equilibrium of hemoglobin, goes back nearly half a century. Actually, what Bohr and his colleagues discovered in 1904 was not the effect of pH but the more restricted effect of carbon dioxide on oxygenation of the blood. Ten years later, in 1914, Christiansen, Douglas and Haldane discovered the converse effect, the effect of oxygenation on the CO equilibrium of the blood. The two effects are, of course, aspects of a single effect, and either one follows thermodynamically from the other in accordance with the general relation

$$\left( \frac{\partial \ln a_1}{\partial m_2} \right)_{m_1} = \left( \frac{\partial \ln a_2}{\partial m_1} \right)_{m_2}$$

where the  $a$ 's denote activities, the  $m$ 's total masses and the subscripts refer to two different components in the system. Later on it came to be realized that the two effects, referred to as the Bohr and the Haldane effects, were not specific to CO<sub>2</sub> but were due to the acid properties of carbonic acid, and it was L. J. Henderson who, in 1920, gave the essentially correct interpretation of it in terms of the presence of a heme-linked or, as we may say speaking in terms of function, oxygen-linked acid group in the molecule (7). It is now known that there are in reality two such groups associated with each heme, one of which is rendered stronger, the other weaker, as a result of oxygenation of the heme and that these are the same for each of the four hemes (6). At 25°C and an ionic strength of 0.16, the pK values of these groups, which we shall refer to as 1 and 2, in horse hemoglobin are estimated as follows (19).

|         | Hemoglobin | Oxyhemoglobin |
|---------|------------|---------------|
| Group 1 | 7.93       | 6.68          |
| Group 2 | 5.25       | 5.75          |

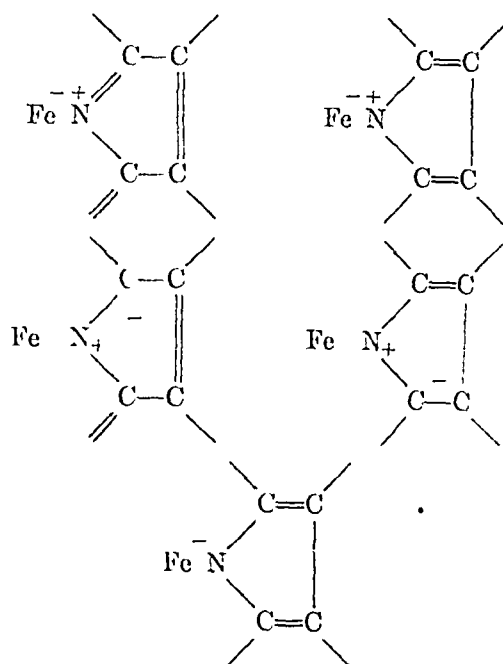
At 37°C all figures are approximately 0.19 unit less. It is group 1 which is accountable for the oxygen Bohr effect in the physiological range, i.e., the one originally discovered. Similar effects, involving other functions, such as oxidation-reduction, and other acid properties have since been revealed.

It is clearly of great interest to identify these two groups. Owing to the range in which they are active and the considerations presented earlier in regard to the buffering power of hemoglobin it is

natural to identify them as imino groups of histidine, and on the basis of structural considerations there is reason to do so, following the original suggestion of Conant (3), though it is, of course, possible that one or the other of them might be one of the free  $\alpha$  amino groups of valine. This suggestion, at least as regards *group 1*, and probably also as regards *group 2*, is strongly supported by thermodynamic reasoning involving the heat of oxygenation of hemoglobin in relation to pH (18). Since, in the region of the Bohr effects, oxygenation is associated with the binding or dissociation of protons, the heat of oxygenation must vary with pH in accordance with the characteristic heat of dissociation of the oxygen-linked acid groups. The value of this heat may, therefore, be obtained from data on the effect of temperature on the differential titration of hemoglobin and oxyhemoglobin, though unfortunately it involves second-order effects in the observations. When this is done the answer comes out as 6500 calories for both groups, though the error in the case of *group 2*, for which the Bohr effect is reduced and which is active in a range where the protein is less stable, is greater than in the case of *group 1*.

Before considering the profound physiological importance of these oxygen-linked groups, presumably histidine, it is worth while to pause to discuss certain structural considerations. These are due mainly to Coryell and Pauling and had their origin in magnetochemical studies on hemoglobin, free heme and certain of their derivatives. In 1936 Pauling and Coryell announced the discovery that hemoglobin was paramagnetic, but that when converted to oxyhemoglobin it became diamagnetic and that the change involved not only the protein but the oxygen molecule as well, which itself became diamagnetic as a result of the reaction (9, 10). In the case of hemoglobin it can be shown that the observed susceptibility corresponds to the presence of four unpaired electrons in each ferrioheme, due to the unaltered electronic configuration of the ferrous ion (15). This can only mean that in hemoglobin the bonding of the iron to the four nitrogen atoms of the porphyrin ring, as well as to the globin, is completely ionic. On combination with oxygen there is a complete change of structure and all these bonds become covalent, like the bond formed with oxygen. As regards the covalent bonds with the four nitrogen atoms of the porphyrin ring,

these may be interpreted as involving a set of resonating structures such as

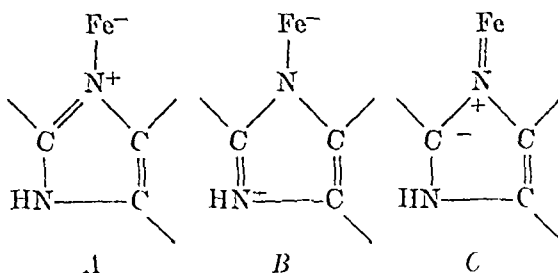


The bond with oxygen may be regarded as a hybrid between



It should be noted that in all these formulae the presence of the bivalent bonds with iron, involving electron pairs drawn from additional third orbitals, reduces the formal negative charge imparted to the iron.

In discussing the non globin bond, Coryell and Pauling (4) assume that there is true bond formation only with the oxygen-linked acid, *group 1*. This they identify as an imidazole group of histidine. In oxyhemoglobin the bond is supposed to involve the following three resonating structures



of which C plays probably a minor rôle. The acid properties of the group are due wholly to form B,

which may be expected to occur with about the same frequency as A. On this basis, by analogy with the pyridinium and free imidazolium ions, Coryell and Pauling estimate a  $pK$  for *group 1* in oxyhemoglobin of about 7. In hemoglobin, with ionic bonding, *form B* will be considerably repressed, and *group 1* should therefore be weakened, as it is known to be. It should be realized that in this heme bonded imidazole from which a proton has been displaced in bond formation, it is the second hydrogen to which the acid properties are due.

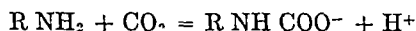
As regards the oxygen-linked *group 2*, it is clear that this cannot be bonded with the iron in oxyhemoglobin, owing to the presence of oxygen. It follows, therefore, from the differential titration data, that it cannot be bonded with it in hemoglobin either, for bonding leads to displacement of a proton. Coryell and Pauling likewise identify this group as an imidazol group and suppose that it is simply electrostatically coordinated with the heme iron (4). In hemoglobin it is thereby strengthened beyond its condition in free histidine (observed  $pK$  5.25 as compared with  $pK$  6 in free histidine). In oxyhemoglobin, due to the shielding influence of the oxygen molecule, it reverts towards its condition in histidine (observed  $pK$  5.75).

The significance of the oxygen linked *group 1* in the exchange of carbon dioxide is most obvious when we consider the case of an individual under conditions where the respiratory quotient is 0.7. Under these conditions there is characteristically no significant change of  $pH$  of cells when venous blood is converted to arterial blood. At physiological reaction (say  $pH$  7.3) we need only consider *group 1*. At this  $pH$  and  $37^\circ C$  the difference in dissociation of this group as between hemoglobin and oxyhemoglobin amounts to almost exactly 0.6 equivalents (per heme). For every mole of oxygen taken up by the blood there must therefore be a loss of 0.6 mole  $CO_2$  in the form of bicarbonate. In addition to this, to keep the  $pH$  of the cells constant, there must also be a loss of approximately 0.07 mole  $CO_2$  in the form of carbonic acid, (taking the dissociation constant of carbonic acid in blood at  $37^\circ C$  as  $5.9 \times 10^{-7}$ ). The sum of these would account for very nearly the complete exchange of carbon dioxide accomplished. On the other hand, when the respiratory quotient approaches unity, both cells and plasma are found to be more alkaline in arterial than in

venous blood (cells about 0.02 unit, plasma about 0.04 unit). This leads to an additional displacement of bicarbonate ion and carbonic acid, chiefly as a result of buffering due to histidine residues in the hemoglobin, which may be calculated as 0.20 to 0.25 mole. Residual amounts of  $CO_2$  exchange may be ascribed to carbamate. The whole process represents a complicated interplay of functions in which, if our identifications are correct, a primary role is played by histidine and for which a detailed mechanism would be provided by the structural picture of Coryell and Pauling.

In conclusion we turn to the question of carbamate formation in the blood, which is closely related to the problem of  $CO_2$  transport and bears upon the structural interpretations which we have just been considering. Our knowledge of this matter comes mainly from the experiments of Ferguson (5) and of Stadie and O'Brien (13). Ferguson worked with unbuffered solutions of human hemoglobin resembling whole blood, Stadie and O'Brien with similar solutions of horse hemoglobin. In the face of severe experimental difficulties both investigators showed that as  $pCO_2$  was added there was first an increase and then a slow decline in carbamate formed, and that when the hemoglobin solutions were oxygenated, carbamate formation dropped, in some of Ferguson's experiments to one third or less of its previous value. Under the conditions of the experiments, the highest values of total carbamate were always much less than 1 equivalent per heme. (In only one observation was it as great as one third). On the basis of his results, Ferguson estimated that as much as 30 per cent of the carbon dioxide transport might be due to carbamate.

Roughton has given an interesting qualitative interpretation of Ferguson's experiments (12). In order to understand this it is necessary to recall certain general facts relating to carbamates. Carbamate formation takes place between the uncharged amino group and dissolved  $CO_2$  (not  $H_2CO_3$ ,  $HCO_3^-$ , or  $CO_3^{=}$ ), and results in loss of a proton, carbamic acids being fairly strong acids ( $pK$  6 or less).



There is no formation of zwitter ions, the  $NH$  group being an extremely weak base due to resonance phenomena. The reaction is limited to aliphatic amines and does not occur with the

imidazole group of histidine. Having regard to these considerations, Roughton arrived at the following picture. The carbamate observed by Feigson resulted from the combination of  $\text{CO}_2$  with free amino groups whose  $\text{pK}$  was in the neighborhood of 7 (Presumably these would have to be free  $\alpha$  amino groups to have such a low  $\text{pK}$ .) The reason why increasing  $\text{pCO}_2$  did not increase the amount of carbamate was because of the effect of increased acidity on the dissociation of these groups. The effect of oxygenation in decreasing carbamate formation was due to the fact that the amino groups were oxygen linked—in fact he identified them with what we have designated as *group 1* and supposed to be histidine. That oxygenation affects the reaction of this group with  $\text{CO}_2$  was explained by steric hindrance.

This view is clearly of great interest in connection with the present discussion. In the first place it would provide a significant additional mechanism for the transport of carbon dioxide, depending merely on the introduction of oxygen into the hemoglobin molecule quite apart from changes in  $\text{pH}$  and  $\text{pCO}_2$ . In the second place, and this apparently has not been noticed previously, it would imply thermodynamically the existence of a significant new mechanism for the exchange of oxygen, for it follows necessarily that if oxygen affects carbamate formation there must be a reciprocal effect of carbamate on oxygenation. Thus, even at constant  $\text{pH}$  and  $\text{pO}_2$ , the formation of carbamate would lead to a loss of oxygen by hemoglobin. It is, however, inconsistent with our identification of *group 1* as an imidazole group of histidine and would necessitate the abandonment of the structural picture of Coryell and Pauling which seems to fit the facts so well. Nor is it so easy to devise an electronic interpretation of the Bohr effect involving *group 1* on the assumption that this is an amino group.

It is of interest to calculate the magnitude of the effect of oxygen on the carbamate equilibrium according to Roughton's hypothesis. For this equilibrium, in accordance with the considerations presented above, we may write

$$\bar{Z} = \frac{K_1 K_2 p}{K_1 K_2 p + K_1 H + H^2}$$

in which  $\bar{Z}$  is the fractional saturation of the amino group with  $\text{CO}_2$ ,  $K_1$  its acid dissociation constant and  $K_2$  the equilibrium constant for its

reaction to form carbamate. Since Roughton postulates that only one amino group per heme is involved,  $\bar{Z}$  may be obtained at once by dividing the measured carbamate by the oxygen capacity of the solution. From the data given by Feigson, including his estimated  $\text{pH}$  values (unfortunately he made no direct  $\text{pH}$  measurements) we calculate in this way  $K_2$  to be between 7 and 8 times greater for hemoglobin than for oxyhemoglobin. This is a minimum figure, if we had assumed other groups, not oxygen linked, to have been involved as well, it would have been larger. The result is significant for it requires as a reciprocal effect that the oxygen affinity of hemoglobin must be between 7 and 8 times that of hemoglobin-carbamate. It suggests a very simple test of the hypothesis which has not yet been tried but which would avoid the severe experimental difficulties involved in direct measurements of carbamate. This would be to study the effect of  $\text{pCO}_2$  on the amount of oxygen combined with hemoglobin at constant  $\text{pH}$  and  $\text{pO}_2$ . Actually, if the measurements were made at  $\text{pH} \geq 9.5$ , where the Bohr effect disappears, it would be unnecessary to maintain strictly constant  $\text{pH}$ , and saturation with  $\text{CO}_2$  should lead to a sevenfold decrease in oxygen affinity.<sup>2</sup> At lower  $\text{pH}$  values it would be necessary to use strongly buffered solutions. By ascribing to the amino group the  $\text{pK}$  values given above for *group 1*, we obtain by simple mass law considerations for  $\text{pH} 7.3$  and for a constant value of  $\text{pO}_2$  such that the solution is 80 per cent saturated with oxygen in the absence of  $\text{CO}_2$ , the ratio  $\frac{d\text{HbO}_2}{d\text{HbCO}_2} \cong -2/3$ .

This value is relatively constant over a wide range of values of  $\text{HbO}_2$  and  $\text{HbCO}_2$ . Experiments of this kind should be relatively easy and decisive.

As a final point in this discussion we offer tentatively the following explanation of the experiments of Feigson and of Stadie and O'Brien as an alternative to that given by Roughton and one which would avoid the difficulties of his hypothesis. Whether or not it is acceptable will depend on an appraisal of the errors of the observations. Instead of thinking in terms of a single heme-linked free  $\alpha$  amino group, let us assume that carbamate formation involves mainly  $\epsilon$  amino groups, whose  $\text{pK}$  values are quite alkali-

<sup>2</sup> The fact that at such an alkaline reaction additional amino groups, say amino groups of lysine, would also take up  $\text{CO}_2$  would make no difference.

line, say considerably greater than 8. These will not of course be oxygen linked. Then for  $pH \ll pK_1$ , equation 1 goes over into  $\frac{\bar{Z}}{1 - \bar{Z}} = \frac{K_1 K_p}{H}$  or, since in accordance with our hypothesis that many groups are involved  $Z \ll 1$ ,  $\bar{Z} = \frac{K_1 K_p}{H^2}$ . Accordingly  $\bar{Z}$  is very sensitive to  $pH$  and the effect of oxygenation in diminishing carbamate formation is to be ascribed simply to the increase of acidity accompanying oxygenation in the unbuffered hemoglobin solutions. Under the conditions of the experiments this may be estimated to be of the order of 0.1. As a test of this hypothesis we have calculated values of  $K_1 K_p$  from the data of Stadie and O'Brien, using their measured  $pH$  values, with the following results:

|                          |        |        |      |      |      |      |      |      |      |
|--------------------------|--------|--------|------|------|------|------|------|------|------|
| <i>Hemoglobin</i>        |        |        |      |      |      |      |      |      |      |
| pCO <sub>2</sub> (mm)    | 0.7    | 2.3    | 10.2 | 47.4 | 139  | 271  |      |      |      |
| $K_1 K_p \times 10^{17}$ | (0.25) | (0.49) | 0.68 | 1.33 | 1.01 | 0.94 | av   | 0.99 |      |
| <i>Oxyhemoglobin</i>     |        |        |      |      |      |      |      |      |      |
| pCO <sub>2</sub>         | 3.6    | 10.6   | 25   | 53   | 96   | 177  | 312  |      |      |
| $K_1 K_p \times 10^{17}$ | 0.66   | 0.57   | 0.59 | 0.77 | 0.97 | 0.80 | 0.50 | av   | 0.69 |

In taking the averages, the two bracketed values for hemoglobin were omitted because of high  $pH$  values, namely 8.52 and 8.15. A similar direct calculation is not possible in the case of Ferguson's data owing to lack of measured  $pH$  values. By making use of calculated  $pH$  values,<sup>3</sup> however, we arrive at the following results for his most complete experiment:

|                          |      |       |       |       |    |      |  |  |  |
|--------------------------|------|-------|-------|-------|----|------|--|--|--|
| <i>Hemoglobin</i>        |      |       |       |       |    |      |  |  |  |
| pCO                      |      |       |       |       |    |      |  |  |  |
| (mm)                     | 4.45 | 19.60 | 43.0  | 80.50 | av | 1.69 |  |  |  |
| $K_1 K_p \times 10^{17}$ | 1.49 | 1.72  | 1.85  | 1.71  | av | 1.69 |  |  |  |
| <i>Oxyhemoglobin</i>     |      |       |       |       |    |      |  |  |  |
| pCO                      |      |       |       |       |    |      |  |  |  |
| (mm)                     | 8.28 | 21.30 | 45.30 | 74.20 |    |      |  |  |  |
| $K_1 K_p \times 10^{17}$ | 0.86 | 1.26  | 1.16  | 1.68  | av | 1.24 |  |  |  |

It is also possible, without assuming an absolute value of  $K_1 K_p$ , to construct theoretical curves

<sup>3</sup> In making the calculations we assume the  $pH$  of one point, take the buffer value of reduced hemoglobin as 2.9 equivalents per  $pH$  unit per heme, employ  $pK$  values of group 1 given above, neglect the very slight effect of group 2 in the  $pH$  range in question, take the solubility of CO in the hemoglobin solutions as  $2.9 \times 10^{-3}$  moles liter<sup>-1</sup>mm<sup>-1</sup> (following Stadie and O'Brien) and (on the basis of the ionic strength of the solution) assume the first dissociation constant of carbonic acid to be  $5.9 \times 10^{-7}$ .

giving relative values of carbamate in relation to  $pCO_2$  on the basis of the same calculations. These curves may then be superposed to best advantage on the experimental points. This has been done for the two experiments just considered and the results are shown in figure 1. This figure also includes values of carbamate calculated from the measured  $pH$  values for Stadie and O'Brien's experiment. It will be seen that there is fair agreement between observed and calculated results, though the calculations cer-

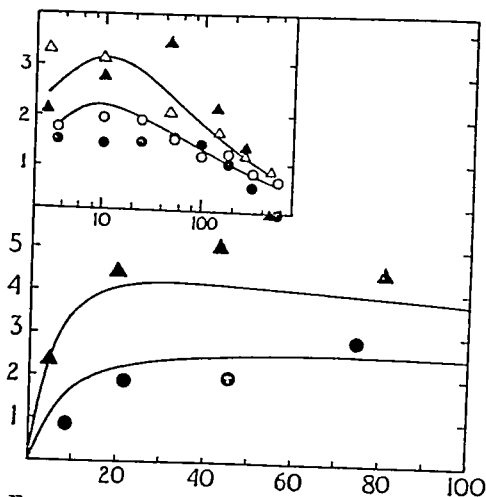


Fig. 1. CALCULATED AND OBSERVED VALUES OF carbamino hemoglobin. Ordinates give  $\bar{Z}$  as carbamino hemoglobin in mm Hg per liter. Abscissae give  $pCO$  (mm Hg). Solid points are observed quantities. Curves are calculated as described in text. Open points are calculated from directly measured  $pH$  values. Triangles are for deoxygenated solutions, circles for oxygenated solutions. Main figure is for Ferguson's most complete experiment (23  $\pm$  35). Insert is for results of Stadie and O'Brien with logarithmic abscissae.

tainly lead to somewhat smaller differences between oxygenated and deoxygenated solutions than correspond to the observations. Whether or not the discrepancy is significant is not certain, and further experiments are certainly in order.<sup>4</sup>

<sup>4</sup> Quite recently it has been pointed out by Professor Roughton (personal communication) that Ferguson's other experiments are less favorable to our interpretation and show a greater difference between  $K_1 K_p$  for hemoglobin and oxyhemoglobin than those just discussed. We have confirmed this using our calculated  $pH$  values. Thus, averaging all Ferguson's results, we obtain for hemoglobin  $K_1 K_p = 1.97 \times 10^{-7}$ , and for oxyhemoglobin  $K_1 K_p = 1.11 \times 10^{-7}$ , but the scatter is very large, extreme individual values varying by a factor of three in each series.

Whichever interpretation proves correct, however, it is clear that carbamate formation, with its possible implications regarding oxygen combination, represents another aspect of the picture we

have sought to develop of hemoglobin as a highly perfected molecular mechanism designed to meet the physiological needs of the vertebrate body

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# MOLECULAR OXYGEN AS A LIGAND IN METAL PORPHYRINS AND OTHER METAL-COMPLEX COMPOUNDS

L. MICHAELIS

*From the Laboratories of The Rockefeller Institute for Medical Research, New York City*

The iron porphyrin compounds occurring in the organism have various functions, all related to oxidation. Warburg's respiratory enzyme activates oxygen, the cytochromes transfer electrons, peroxidases activate hydrogen peroxide, catalase destroys it, hemoglobin carries molecular oxygen. Of all these functions, the most marvellous one, the one least imitable in model experiments, seems to be the ability of hemoglobin to carry oxygen, reversibly absorbing or releasing it, in response to minor variations in oxygen pressure. There is no satisfactory answer as to how this function of hemoglobin can be accounted for by its structure. The task of this paper is to discuss a number of facts, theories and hypotheses, some old and almost forgotten, others more recent, which may throw some light on this problem and may stimulate further attempts in solving this riddle. Since the whole topic is concerned with coordinative metal complex compounds, a few remarks about some general aspects of metal complex compounds will serve as an introduction.

It has been recognized for a long time that metal complex compounds may be classified in two groups according to the nature of the bond by which the ligands are bound to the central metal atom. Those two kinds were distinguished in the older German chemical literature as 'Anlagerungscomplexe' (complexes by juxtaposition) and 'Durchdringungscomplexe' (complexes by penetration). This distinction can be expressed in modern terms as follows. In one type of complex the ligands are held by electrostatic forces exerted by a positively charged metal ion upon ligands which are either negative ions with a free charge or dipoles, either permanent or induced. For instance, among the ligands frequently encountered, water is a distinct permanent dipole, ammonia is a weaker permanent dipole but it is easier to polarize. Such bonds, in Pauling's (1) nomenclature, are called ionic bonds. They are also called electrovalent, or heteropolar bonds. An example of an ionic complex is the ferric hexafluoride ion,  $\text{Fe}^{\text{III}}\text{F}_6^-$ , where six fluorine ions are coordinated around one triply positively-charged ferric ion. Electrostatic forces impose no

definite restriction on the number or spacial configuration of the ligands, but spacial restrictions limit this number to six which will form the corners of an octahedron. The electronic configuration of the atoms within this complex is not essentially disturbed. Ferric ion, both in the state of the free (or hydrated) ferric ion, and of this complex ion, has five unpaired electrons in its incompleated shell (the 3d sub shell). This conclusion is derived from the fact that the magnetic susceptibility is the one theoretically expected for the presence of five unpaired electrons in either case. The other type of complex compound is characterized by an essential change of the electronic configuration. In the ferrocyanide ion  $\text{Fe}^{\text{II}}(\text{CN})_6^-$  there are no unpaired electrons at all, this complex ion is diamagnetic, although ferrous ion in the free state is strongly paramagnetic due to four unpaired electrons in the 3d sub shell. Such compounds may be formed with such ligands which possess electrons not used for chemical bonding. The electrons of the ligands are used for the completion of the uncompleted electron shell of the metal ion so as to approach the structure of a noble gas (krypton in this case). The six ligands occupy the corners of a regular octahedron not only for spacial reasons but because the covalent bonds have the greatest stability in such a configuration, as Pauling has shown by quantum mechanical calculations. In his nomenclature all such bonds are called *covalent* bonds, or they may be called also *homopolar* bonds. In the ferricyanide ion  $\text{Fe}^{\text{III}}(\text{CN})_6^-$  there is an odd number of electrons. Here the approach to a noble gas structure is less complete because one electron must be left unpaired whereby the paramagnetism of the ferric ion is considerably weakened indeed but not entirely abolished.

In complexes of iron it is easy to infer from magnetic measurements whether a complex belongs to the one or the other type. This is not necessarily so for all metals. So, any cupric compound will always show a magnetic susceptibility corresponding to one unpaired electron no matter what type of bond is established.

Some ligands are more inclined to form ionic bonds, others, covalent bonds. The strongly 'electronegative' fluorine ion always forms ionic bonds, cyanide ion always forms covalent bonds.

Water is usually coordinated by ion dipole bonds, but in some cobaltic complexes by a covalent bond. Ammonia is usually bound by electrostatic bonds in cobaltous complexes, but by covalent bonds in cobaltic complexes. In hemoglobin the bonds of the iron with the N-atoms of the porphyrin ring are ionic, leaving undisturbed the paramagnetism of the central ferrous ion with its four unpaired electrons, whereas in oxyhemoglobin all bonds are covalent, destroying all paramagnetism, as Pauling and Coryell (2) have shown.

In general, the two types of bond are but extreme cases. There are many transitions which may be imagined to arise from resonance, each of the extreme configurations contributing a certain share to the actual, intermediate state. However, as regards the complex compounds of iron, cobalt and some other metals, we are faced with a peculiar situation which will now be discussed. Let us consider for instance the case of hemoglobin. How can it be explained that hemoglobin has a magnetic susceptibility as high as is to be accounted for by purely ionic bonds, with four unpaired electrons in the iron atom in its ferrous state, whereas oxyhemoglobin, which is also in the ferrous state, has no unpaired electrons at all, is diamagnetic, and shows only covalent bonds with no intermediate or resonating condition occurring? The explanation is this. In a complex ion compound the ionic form of the complex has a different number of unpaired electrons than has the covalent form. According to the rules of quantum mechanics, resonance between states possessing a different number of unpaired electrons cannot take place. On resorting to this rule, one may assume that a resonance between the two states does not occur in an iron complex compound. The molecule has to stay in one of the two possible states, whichever is the more stable one, even if the difference in stability be small. It is quite possible that even in such cases intermediate bonds do exist, as Pauling has pointed out. So, the ionic complex may be in resonance with a covalent one, not of the ordinary type but one formed by 'promoted' energy levels of some electrons in such a way that the number of unpaired electrons is not affected. Such covalent bonds, if they exist, will be weaker than regular ones, furthermore, they would permit of resonance. For this reason Pauling prefers to speak of an 'essentially' ionic, or covalent, bond in such cases. It will cause no misunderstanding if this fine distinction is disregarded in what follows. The essence of this consideration is that if there is a choice between two magnetic states, the

state will be either the one, or the other, according to conditions, but never an intermediate state. If we accept this argument it will be easier to understand why such a slight perturbation as the attachment of an oxygen molecule in hemoglobin is able to throw the magnetic character of the complex entirely from one extreme to the other.

It is an essential gap in the present theory of coordination compounds that it is, in general, not possible to predict whether a ligand will form an ionic or a covalent bond. Of course, if a ligand is neither an ion nor a distinct dipole, it can form only a covalent bond. This is true, e.g., for molecular oxygen. If we accept this definition we see that it is not always true that a covalent bond is necessarily a strong or 'robust' bond.

The bond with molecular oxygen can be established in two different ways for both of which examples may be cited. The molecule  $O_2$  contains an even number of electrons. However, the ground state of  $O_2$  contains two unpaired electrons, which makes  $O_2$  paramagnetic. Oxygen can be attached as a ligand either in such a way that after pairing of those two electrons, a pair of electrons is used in filling up the incomplete electron shell of the central metal atom, thus forming a 'dative bond', (as in oxyhemoglobin and in one type of the cobalt compounds to be discussed presently), or, in a binuclear complex with an  $O_2$  bridge, each one of the unpaired electrons of  $O_2$  may form a pair with one of the unpaired electrons of the central metal atom (as in another type of cobalt complex). In any case, oxygen, although it is itself, in the free state, paramagnetic, will, as a ligand, always diminish and usually even annihilate the paramagnetism of the complex compound.

Among other gases, CO and NO can function as ligands. In some cases, each of the three gases can be bound, as in hemoglobin. In other cases,  $O_2$ , but not CO, can be bound. CO may sometimes occupy many or all coordination places as in the metal carbonyls. Oxygen seems to be capable of coordination only in such cases where other ligands have been already bound. It is not unusual in coordinative compounds that the affinity of a ligand is strengthened if there are other ligands of a different kind already attached.

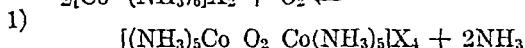
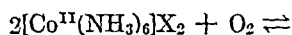
Coordination compounds containing molecular oxygen are stable compounds only in a few rare cases. Usually they are inclined to a more or less rapid intramolecular rearrangement whereby oxygen is reduced and something else, usually the central metal atom, is oxidized. One may speak

of an intramolecular redox system. This intramolecular reaction is in many cases so rapid that the existence of the original, oxygenated complex escapes direct observation and only the result of the interaction can be noticed. In some cases the intramolecular rearrangement stops with the oxidation of the metal atom. For instance, the cobaltous bicycsteme complex (3) is oxidized by oxygen to a cobaltic complex of cysteme and not readily any further. The reason is that in a cobalt complex compound, once the cobaltic state has been established it is very difficult to reduce it back to the cobaltous state. In other cases, a chain reaction is started and the metal atom may be said to activate molecular oxygen as an oxidizing agent. For instance, in the ferrous bicycsteme complex the oxidation goes farther, cysteme being oxidized by molecular oxygen to cysteine with very little iron as a catalyst. Primarily, just as in the case of the cobalt cysteine complex, ferrous cysteine is oxidized by oxygen to ferric cysteine, then an intramolecular oxidation-reduction process leads to ferrous iron and cysteine, the ferrous iron combines with more cysteine, and a chain reaction is started. However, the very primary step in this process is the incorporation of an oxygen molecule into the ferrous cysteine complex, although this oxygenated complex is so labile that it cannot be prepared as such. As to the structure of this hypothetical labile oxygenated ferrous bi-cysteine complex, it may contain one molecule of oxygen per one atom of iron, or, perhaps in analogy to some compounds to be discussed presently, one oxygen molecule may form a bridge between two molecules of the ferrous complex, and a binuclear complex may be formed. The hypothesis that primarily an oxygenated ferrous complex is formed is corroborated by the fact that also carbon monoxide can be bound as a ligand, and this carbon monoxide ferrous cysteine complex can be prepared in pure, crystalline form.

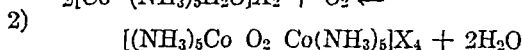
Reversible oxygenation, without any intramolecular irreversible rearrangement occurring, is encountered among the iron compounds only in hemoglobin. Cases more or less comparable are known only in cobalt compounds. The discovery of such cobalt compounds has aroused the hope of finding out under what conditions a reversible oxygenation can take place. What is known now in this field will now be briefly discussed.

The first case of this kind has been known for more than fifty years, but has been almost forgotten, or at least never been discussed as a model

for oxygenation. It is concerned with the hexammine cobaltous ion,  $\text{Co}^{II}(\text{NH}_3)_6^{++}$ . It reacts with molecular oxygen. However, the effect of oxygen is not, or at least not primarily, the oxidation to the corresponding cobaltic compound, which is the luteo cobalt ion, but rather is the first product of interaction readily obtainable in pure crystalline form, of intensely brown color, a compound formerly called 'oxycobaltiac'. The reaction was explained by Werner (4) as follows:



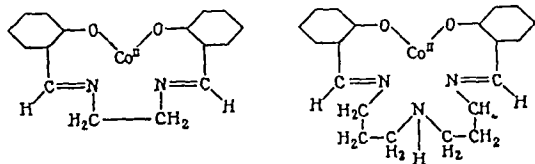
where  $X$  is an univalent anion. Since in the dissolved state the hexammine cobaltous complex, even in the presence of excessive ammonia, is always in equilibrium, at least with the pentammine, one may just as well modify Werner's formula as follows:



Werner and Mylius (5) strongly suggest the reversibility of this reaction although they did not succeed in proving it experimentally for this particular case. This compound, easy to prepare and quite stable in the crystalline state, is readily decomposed by water or acids, with sudden release of all of its oxygen as gas. This fact seems in favor of assuming what we may call a cobaltous compound in the oxygenated state, although this evidence is not quite convincing because cobaltic ion,  $\text{Co}^{+++}$ , is present in cobaltic sulfate, also decomposes water with the development of oxygen, the normal potential of the couple, cobaltous ion + cobaltic ion, lying in the oxygen overvoltage range. However, as will be published separately, it is easy to arrange the experimental conditions in such a manner that the 'oxycobaltiac' can be reversibly oxygenated and deoxygenated according to the oxygen pressure, just as with hemoglobin. In the dissolved state the effect of oxygen does not stop at this stage, rather is there very gradually established a true oxidation of the cobalt. A mixture of pentammine aquo cobaltic salts (roseo cobalt salts) and some hexammine cobaltic salts (luteo cobalt salts) is eventually established. So, the oxycobaltiac may be considered as a precursor, although a relatively stable one, to a true oxidation product. Anyhow, molecular oxygen does not directly oxidize the cobaltous state to the cobaltic by simply withdrawing one electron. Such a reaction can be

accomplished by other oxidizing agents. In this particular case a potentiometric oxidative titration of the cobaltous compound with ferricyanide does not lend itself to readily interpretable results for reasons which have been fully explained by J. Bjerrum (6). However, on using the much more chelated, strongly coordinated ethylene diamine complex instead of the ammonium complex, Bjerrum has shown that the potentiometric titration of the cobaltous complex with potassium ferricyanide yields a titration curve which precisely in every respect is characteristic of a reversible, univalent, true oxidation. Thus we can distinguish, just as in the case of hemoglobin, oxygenation as an effect of oxygen, and a true oxidation as an effect of potassium ferricyanide.

Another group of oxygenated cobaltous complexes was extensively studied by Calvin and his associates (7, 8). After Tsumiki (9) had discovered that a certain chelated cobaltous complex can reversibly absorb or release oxygen in the same manner as can hemoglobin, Calvin prepared a large number of cobaltous complexes with the



same property. They are all chelated complexes in which one or two coordination places are unoccupied. He distinguishes two types, of which the simplest representatives are shown in formulas 1 and 2. All rings established by chelation are 5- or 6-membered rings. They are prepared from a) a cobaltous salt, b) salicylic aldehyde or related compounds, c) ethylene diamine or (in type 2) a related compound. These compounds absorb molecular oxygen with the development of an intense brown color, in a reversible way. The oxygen can be pumped out or released by heating, or by acidification. The amount of oxygen absorbed depends on pressure and temperature, an equilibrium being attained as in hemoglobin. The absorption of oxygen takes place in a suitable organic solvent, or even in the solid crystalline state. The reversibility is not always quite complete. After several cycles of absorption and desorption, usually a deterioration takes place, which is at least in part some intramolecular oxidation, which however does not seem to be simply the oxidation of the cobaltous state to the cobaltic. In the 3-fluoro derivative of the complex

of type I, no deterioration takes place even after many cycles, and this compound as a solid can be used for storage of oxygen by absorbing it from the air at room temperature and releasing it at higher temperature. Complexes of type I absorb one O<sub>2</sub> molecule for two cobalt atoms, the oxygen forming a bridge in a binuclear complex as in the oxycobaltic mentioned before. Complexes of type II absorb one O<sub>2</sub> molecule per one cobalt atom. The magnetic properties are these. Complexes of type II have three unpaired electrons, just as the free cobaltous ion, and in the oxygenated state, the electrons are paired as much as possible, leaving one unpaired electron. In type I the cobaltous complex has only one unpaired electron, and the oxygenated complex is diamagnetic, which shows that all electrons are paired, especially also that each of the two unpaired electrons as they exist in the free O<sub>2</sub> molecule has paired with one electron of cobalt. There are then two types with respect to magnetic properties, to which we shall presently add a third one.

Another class of cobaltous complexes capable of reversible oxygenation has been discovered by Burk, Heaton, Caroline and Schade (10), namely, the cobalt complexes with some amino acids, especially with histidine. Here the resemblance to hemoglobin is even closer as the oxygenation takes place in an aqueous solution. The non-oxygenated complex is paramagnetic with three unpaired electrons, the oxygenated one, which is binuclear with an O<sub>2</sub> bridge, is diamagnetic (11), and so represents a third magnetic type, in addition to the two described by Calvin. The oxycobaltic mentioned before also belongs to this class. In the oxygenated state it is diamagnetic, as stated already in 1911 by Feyt's (12), and corroborated by the speaker, whereas all cobaltous complexes are paramagnetic with three unpaired electrons.

The reversible oxygenation of cobalt complexes with amino acids was entirely overlooked during the most interesting previous studies (13). Although oxidative changes of the cobaltous complexes on exposure to oxygen had been noticed, well defined compounds were obtained only by heating a suspension of cobaltic hydroxide with amino acids such as glycine or alanine. The surprising discovery of the various kinds of stereoisomers and their enormous rotatory power was apt to push into the background the reactions of the corresponding cobaltous compound with oxygen, seemingly resulting in an undecipherable

mixture of various compounds. This problem was studied, only recently, by Burk *et al.*, (10) especially for the case of the cobaltous bi-histidine complex. When this complex of light pink color is exposed to the air, it absorbs oxygen, the color turning intensely brown. This reaction is reversible: oxygen is released by pumping it out, or at high temperature, or on the decomposition of the complex by acids. The oxygenated cobaltous bi-histidine complex contains one  $O_2$  for two Co atoms and is obviously a binuclear complex resembling the oxycobaltic mentioned above. This oxygenated complex is not very stable. Gradually it undergoes an irreversible rearrangement, turning pink. According to Burk and associates, it absorbs another molecule of oxygen without there being any evidence for the oxidation of the cobaltous state to the cobaltic. Although those secondary reactions have not yet been studied in detail and no pure compounds have been reported so far, I wish to mention that according to experiments by Dr. S. Fiala in my laboratory, the first step of the irreversible reaction which establishes the light pink compound takes place before any additional oxygen is absorbed. What is interesting at this point is only the fact that there is evidence for the existence of a reversibly oxygenated complex, which slowly undergoes an electronic rearrangement whereby its reversibility is lost.

What is true for histidine can be shown to be true also with some other amino acids, although the affinity for oxygen is much smaller. What seems to be necessary is the formation of a complex with two molecules of the amino acid, fortified by strong chelation and the formation of five or six-membered rings. In this respect histidine is, if not unique, yet especially favorable because it provides for strong chelation with six-membered rings through both its amino-nitrogen and the ring nitrogen.

An important problem, however, is why such a remarkable difference with respect to the stability of the oxygenated complexes prevails between iron and cobalt. No satisfactory answer can be given, yet certain facts may be correlated with this difference. There is always a reversible transition from the ferric state to the ferrous. The normal potential of the ferric-ferrous couple for various complex compounds does vary indeed, but within reasonable limits (rarely  $> 0.7$  volts, or  $< -0.2$  volts) (14). However, the normal potential of the cobaltic-cobaltous couple varies within enormous limits indeed. The potential of

the couple, hexacyano cobaltous and cobaltic, complex is in the hydrogen overvoltage range, the cobaltous compound reduces water to hydrogen. The potential of the couple of the free (or hydrated) cobaltic and cobaltous ion is in the oxygen overvoltage range, cobaltic ion develops oxygen from water. On considering the electronic configuration, it has been made plausible by J. L. Hoard, according to a quotation in Pauling's book (1, p. 93), why cobalt complexes, provided they are covalent, should be unstable in the cobaltous form. However, it is not clear why the transition from the cobaltous state to the cobaltic is so difficult to bring about with oxygen as an oxidizing agent, in spite of the fact that oxygen so readily enters into the complex. There is obviously a high activation energy for the intramolecular oxidation-reduction. For the biochemist the cobalt compounds are interesting mainly because they show the existence of oxygenated compounds and encourage the hypothesis that oxygenated iron compounds also exist, although they are just short-lived precursors of intramolecular true oxidation with the exception of the case of hemoglobin.

Finally, it is worth while discussing why molecular oxygen is usually inert and needs activation at all. Only the principle of obligatory univalent oxidation (15) can explain the inertia of molecular oxygen. This principle may be stated as follows: Whenever an oxidation takes place in solution by collision of the oxidizing molecule with the molecule to be oxidized, without the formation of a complex compound of the two, only a univalent oxidation, a transfer of a single electron, has any appreciable chance to occur. All bivalent or polyvalent oxidations proceed in univalent steps, which may more or less overlap. If the free energy involved in the first univalent step is high, this energy step may be considered as the essential part of the 'activation energy' for the overall, bivalent oxidation. In oxygen, the first step of reduction must be  $O^-$ , designated as 'superoxide ion' (1, 8), or, in aqueous solution,  $O_2H$ , a radical, which Latimer (16, 17) has termed 'perhydroxyl'. Estimates about the oxidation potential of the couple  $O/O^-$  have been made by Latimer (16, 17) and by Gorm (18). Such a potential, or the free energy equivalent to it, depends on the concentrations and states of the molecular species concerned. What is usually called the 'normal potential' is related to a 'standard condition', which is difficult to define for a molecular species.

such as  $O_2^-$ , especially if the electron transfer takes place within the molecule of the complex compound. For this reason the normal potential of the couple  $O_2, O_2^-$ , as calculated by the authors mentioned, is not of much avail. However, qualitatively at least the principle may be stated as follows. The energy necessary to bring about the first step in the reduction of oxygen,  $O_2 + e \rightarrow O_2^-$  is rather high for the free oxygen molecule. When  $O_2$  is a constituent of an oxygenated metal complex, this energy may be quite different. In an oxygenated ferrous complex, the energy of the intramolecular reaction  $(O_2, Fe^{++}) \rightarrow (O_2^-, Fe^{++})$  or, if the complex be binuclear,  $(Fe^{++}, O_2, Fe^{++}) \rightarrow (Fe^{+++}, O_2^-, Fe^{+++})$  is small and the process will readily proceed. Whereas the energy of the analogous intramolecular reaction with cobalt is very high, the reaction will not readily proceed in spite of the fact that in many cobalt complex compounds the cobaltic state, once it is established, is much more stable than the cobaltous one, and therefore the oxidation of most cobaltous complexes to the cobaltic state can be brought about with great ease with other oxidizing agents which do not require much activation energy, such as potassium ferricyanide.

Among the various, mostly unknown factors which may influence the activation energy of molecular oxygen, it may be justified to indulge in the following speculation. If an oxygen molecule forms a bridge in a binuclear complex, there is some chance that if one electron happens to jump from one ferrous atom to  $O_2$ , almost simultaneously another electron may jump from the other ferrous atom also to  $O_2$ . In this case the oxidation level of  $O_2^-$  or  $H_2O_2$  can be reached with much greater ease, since  $H_2O_2$  is very much more stable than  $O_2$ . In the same manner,  $H_2O_2$  as a bridge, could be reduced directly to  $H_2O$ . One may wonder whether the function of the specific protein, globin in hemoglobin, is to fix the position of the heme molecules and keep them apart from each other so that an oxygen molecule attached to one of them can never form a bridge to another. This would be at least one factor, among others unknown, in stabilizing oxyhemoglobin and prevent oxygen from oxidizing it to methemoglobin, in contrast to all hematochromogens, including denatured hemoglobin, which are readily oxidized to the ferric state by molecular oxygen.

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## Thirty-Third Annual Meeting

### DETROIT, MICHIGAN

#### *April 18-22, 1949*

The 1949 convention of the Federation will be held in Detroit, Michigan, April 18-22. The scientific sessions of the six constituent Societies will begin at 9 00 A M, Tuesday, April 19, in the Masonic Temple. They will continue through Friday afternoon, April 22. Sunday and Monday, April 17 and 18, will be devoted to meetings of the Society Councils and of the Federation Executive Committee. The Federation Joint Session will be Tuesday evening, April 19, at 8 00 P M, in the auditorium of the Masonic Temple.

*Registration* will open at 9 00 A M, Monday, April 18, at the Masonic Temple. The registration desks will be open until 10 00 P M Monday evening, and from 8 00 A M to 5 00 P M on Tuesday, Wednesday, Thursday and Friday. Since the scientific sessions begin at 9 00 A M on Tuesday, members are urged to complete registration on Monday, if possible. Members of any of the constituent Societies, guests, and other biologists and physicians who wish to attend the meetings may register. The registration fee will probably be \$6 00. The official badge, issued at registration, must be worn to secure admission to the scientific sessions and other activities of the Convention. There will be a separate registration desk where ladies who are present as guests may register without charge. Programs, abstracts and tickets for various special functions will be on sale near the registration desk.

*Headquarters Hotels* will be the Book Cadillac Hotel for the American Society for Experimental Pathology, the American Society of Biological Chemists and American Association of Immunologists, and the Statler Hotel for the American Physiological Society, the American Society for Pharmacology and Experimental Therapeutics and the American Institute of Nutrition.

*Hotel Reservations* should be made direct to the hotel of choice. Forms for this purpose have been distributed to members through the Society Secretaries and should be filled out and sent in at the earliest possible time. Hotel facilities in Detroit are adequate but the Headquarters Hotels (Book-Cadillac and Statler) cannot accommodate all of the Federation. Approximate rates will be from \$3 50 to \$6 00 for a single room, \$6 00 to \$9 00 for a double room and \$6 50 to \$10 50 for a twin bed room.

An *Informal Mixer* is planned for Wednesday evening, April 20, from 10 00 P M to midnight. The official badge will be necessary for admission and all who have registered are cordially invited to attend. The Mixer will be in one of the Headquarters Hotels.

*Exhibits* There will be no static demonstrations at the Convention. A commercial exhibit, held for the first time in Atlantic City in 1948, is planned again for the Detroit meeting. Exhibitors will be publishers and manufacturers of equipment, apparatus, supplies, chemicals and pharmaceuticals. The exhibit will be in the Masonic Temple.

*Motion Pictures* will be shown at one session, to be scheduled in the program. The title and abstract of the film must be submitted to the Society Secretaries. Only 16 mm safety film can be shown, equipment for sound projection will be available.

*Dinners and Luncheons* Facilities will be ample for holding group dinners and luncheons. Members desiring to schedule these special functions should make their plans early and inform the Federation Secretary (Dr M O Lee, 2101 Constitution Ave., Washington 25, D C) of their needs by March 1, 1949. Specifications should include attendance expected, desired time, and whether or not motion pictures or slides will be shown.

*Local Committee* The Local Committee includes Dr Arthur H Smith, (Wayne University School of Medicine, Detroit 1), Chairman, and Drs E F Beach, O H Gaebler, F W Hartman, D M McGinty and J M Orten.

*Placement Service* It is planned to have an office of the Placement Service of the Federation open in the Masonic Temple during the meetings. Those planning to use its services are requested to submit to the Federation Secretary, not later than April 1, 1949, a résumé of their education, training, experience, publications and type of position desired, together with any other pertinent information. Additional copies of this résumé should be brought to the Convention.

Programs, abstracts and additional announcements will appear in the March 1949 issue of *FEDERATION PROCEEDINGS*.

## EXECUTIVE COMMITTEE 1948-1949

MAURICE B VISSCHER, D B DILL, American Physiological Society  
 HANS T CLARKE, OTTO A BLISSEY, American Society of Biological Chemists  
 CARL A DRAGSTEDT, HARVEY B HAAG, American Society for Pharmacology and  
 Experimental Therapeutics  
 H P SMITH, FRIEDA S ROBSCHLEIT-ROBBINS, American Society for Experimental Pathology  
 E M NELSON, J H ROE, American Institute of Nutrition  
 MICHAEL HEIDLLBERGER, JULES FREUND, American Association of Immunologists  
 H P SMITH, *Chairman*, Columbia University, College of Physicians and Surgeons, New York City  
 MAURICE H SLEAFERS, *Ex Chairman*  
 M O LEL, *Federation Secretary*, 2101 Constitution Ave., Washington, D C

### STANDING COMMITTEES

*Promotion of Biological Research* A C IVY, *Chairman*, K F MEYER, EPHRAIM SHORR  
*International Congresses* D W BROWN, *Physiology, Chairman*, A J CARLSON, *Physiology*, D D VAN SLIJK, *Biochemistry*, H B VAN DYKE, *Pharmacology*, PLYTON ROUS, *Pathology*, L A MAYNARD, *Nutrition*, J J BRONFENBRENNER, *Immunology*  
*Placement Service* M O LEL, *Director*  
*Representatives, Council* A A A S G PHILIP GRABFIELD, C GLEN KING  
*Federation Proceedings, Control Committee* WILLIAM H CHAMBERS, *Chairman*, *Physiology*, ERIC G BALL, *Biochemistry*, MCKEEN CATTELL, *Pharmacology*, MORTON MCCUTCHEON, *Pathology*, A H SMITH, *Nutrition*, A P LOCKE, *Immunology*

### FORMER EXECUTIVE COMMITTEES

Philadelphia, Dec 28-31, 1913

S J MELTZER, *Chairman*, and A J CARLSON, *Secretary*, The Physiological Society A B MACALLUM and P A SHAFFER, The Biochemical Society T SOLLMANN and J AUER, The Pharmacological Society

St Louis, Dec 27-30, 1914

G LUSK, *Chairman*, and P A SHAFFER, *Secretary*, The Biochemical Society T SOLLMANN and J AUER, The Pharmacological Society R M PLARCE and G H WHIPPLE, The Pathological Society W B CANNON and A J CARLSON, The Physiological Society

Boston, Dec 26-29, 1915

TORALD SOLLMANN, *Chairman*, and JOHN AUER, *Secretary*, The Pharmacological Society THEOBALD SMITH and PEYTON ROUS, The Pathological Society W B CANNON and C W GREENE, The Physiological Society WALTER JONES and P A SHAFFER, The Biochemical Society

New York, Dec 27-30, 1916

SYMON FLEXNER, *Chairman*, and PEYTON ROUS, *Secretary*, The Pathological Society W B CAN-

NON and C W GREENE, The Physiological Society WALTER JONES and STANLEY R BENEDICT, The Biochemical Society REID HUNT and J AUER, The Pharmacological Society

Minneapolis-Rochester, Dec 27-29, 1917

FREDERIC S LEE, *Chairman*, and CHARLES W GREENE, *Secretary*, The Physiological Society CARL L ALSBERG, and STANLEY R BENEDICT, The Biochemical Society REID HUNT and L G ROWNTRELL The Pharmacological Society LUDWIG HEKTOLN and HOWARD T KARSNER, The Pathological Society

Baltimore, April 24-26, 1918

CARL L ALSBERG, *Chairman*, and STANLEY R BENEDICT, *Secretary*, The Biochemical Society REID HUNT and E D BROWN, The Pharmacological Society H GIBSON WILLS and HOWARD T KARSNER, The Pathological Society FREDERIC S LEE and CHARLES W GREENE, The Physiological Society

Cincinnati, Dec 29-31, 1919

A S LOEVENHART, *Chairman*, and E D BROWN, *Secretary*, The Pharmacological Society W G MACALLUM and HOWARD T KARSNER, The Pathological Society WARREN P LOMBARD and CHARLES W GREENE, The Physiological Society STANLEY R BENEDICT and VICTOR C MYERS, The Biochemical Society

Chicago, Dec 28-30, 1920

WILLIAM H PARK, *Chairman*, and HOWARD T KARSNER, *Secretary*, The Pathological Society WARREN P LOMBARD and CHARLES W GREENE, The Physiological Society STANLEY R BENEDICT and VICTOR C MYERS, The Biochemical Society A S LOEVENHART and EDGAR D BROWN, The Pharmacological Society

New Haven, Dec 28-30, 1921

J J MACLEOD, *Chairman*, and CHARLES W GREENE, *Secretary*, The Physiological Society D D VAN SLIJK and VICTOR C MYERS, The Biochemical Society C W EDMUNDS and EDGAR D BROWN, The Pharmacological Society, F G NOVY and WADE H BROWN, The Pathological Society



**Toronto, Dec 27-29, 1922**

D D VAN SLIKE, *Chairman*, and VICTOR C MYERS, *Secretary*, The Biochemical Society C W EDMUNDS and EDGAR D BROWN, The Pharmacological Society HOWARD T KARSNER and WADE H BROWN, The Pathological Society, J J R MACLEOD and CHARLES W GREENE, The Physiological Society

**St Louis, Dec 27-29, 1923**

C W EDMUNDS, *Chairman*, and EDGAR D BROWN, *Secretary*, The Pharmacological Society E L OPIE and WADE H BROWN, The Pathological Society A J CARLSON and CHARLES W GREENE The Physiological Society PHILIP A SHAFFER and VICTOR C MYERS, The Biochemical Society

**Washington, Dec 29-31, 1924**

ALFRED S WARTHIN, *Chairman*, and E B KRUMBHAAR, *Secretary*, The Pathological Society A J CARLSON and WALTER J MEEK, The Physiological Society, P A SHAFFER and D WRIGHT WILSON, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society

**Cleveland, Dec 28-30, 1925**

A J CARLSON *Chairman*, and WALTER J MEEK, *Secretary*, The Physiological Society H C SHERMAN and D WRIGHT WILSON, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society GEORGE H WHIPPLE and E B KRUMBHAAR, The Pathological Society

**Rochester, N Y, April 14-16, 1927**

E C KENDALL, *Chairman*, and F C KOCH *Secretary*, The Biochemical Society JOHN AUER and E D BROWN, The Pharmacological Society W H BROWN and E B KRUMBHAAR, The Pathological Society J ERLANGER and W J MEEK, The Physiological Society

**Ann Arbor, April 12-14, 1928**

CARL VOEGTLIN, *Chairman*, and E D BROWN, *Secretary*, The Pharmacological Society DAVID MARINE and CARL V WELLER, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society

**Boston, Aug 19-24, 1929**

(The XIIIth International  
Physiological Congress)

EDWARD B KRUMBHAAR, *Chairman*, and CARL V WELLER, *Secretary*, The Pathological Society JOSEPH ERLANGER and WALTER J MEEK, The Physiological Society E V MCCOLLUM and D WRIGHT WILSON, The Biochemical Society CARL

VOEGTLIN and E D BROWN, The Pharmacological Society

**Chicago, March 26-29, 1930**

WALTER J MEEK, *Chairman*, and ALFRED C REDFIELD, *Secretary*, The Physiological Society W R BLOOR, and HOWARD B LEWIS, The Biochemical Society CARL VOEGTLIN and E D BROWN, The Pharmacological Society WILLIAM F PETERSEN and CARL V WELLER, The Pathological Society

**Montreal, April 8-11, 1931**

W R BLOOR, *Chairman*, and H B LEWIS, *Secretary*, The Biochemical Society GEORGE B WALLACE and E D BROWN, The Pharmacological Society FREDERICK L GATES and C PHILIP MILLER, The Pathological Society WALTER J MEEK and ARNO B LUCKHARDT, The Physiological Society

**Philadelphia, April 27-30, 1932**

GEORGE B WALLACE, *Chairman*, and V E HENDERSON, *Secretary*, The Pharmacological Society SAMUEL R HAYTHORN and C PHILIP MILLER, The Pathological Society WALTER J MEEK and ARNO B LUCKHARDT, The Physiological Society H C BRADLEY and HOWARD B LEWIS, The Biochemical Society

**Cincinnati, April 10-12, 1933**

PEYTON ROUS, *Chairman*, and C PHILIP MILLER, *Secretary*, The Pathological Society ARNO B LUCKHARDT and FRANK C MANN, The Physiological Society H C BRADLEY and HOWARD B LEWIS, The Biochemical Society WM DEB MACNIDER and V E HENDERSON, The Pharmacological Society

**New York, March 28-31, 1934**

ARNO B LUCKHARDT, *Chairman*, FRANK C MANN, *Secretary*, and ALEXANDER FORBES, *Treasurer*, The Physiological Society W M CLARK and H A MATTILL, The Biochemical Society W DEB MACNIDER and V E HENDERSON, The Pharmacological Society CARL V WELLER and C PHILIP MILLER, The Pathological Society

**Detroit, April 10-13, 1935**

W M CLARK, *Chairman*, H A MATTILL, *Secretary*, and C H FISKE, *Treasurer*, the Biochemical Society CHARLES W GREENE and FRANK C MANN, The Physiological Society R A HITCHER and E M K GEILING, The Pharmacological Society S BURT WOLBACH and SHIELDS WARREN, The Pathological Society

**Washington, March 25-28, 1936**

V E HENDERSON, *Chairman*, E M K GEILING, *Secretary*, and C M GRUBER, *Treasurer*, The

Pharmacological Society FRANK C MANN and ANDREW C IVY, The Physiological Society H B LEWIS and H A MATTILL, The Biochemical Society OSKAR KLOTZ and SHIELDS WARREN, The Pathological Society

#### Memphis, April 21-24, 1937

ALPHONSE R DOCHEZ, *Chairman*, and SHIELDS WARREN, The Pathological Society FRANK C MANN and ANDREW C IVY, The Physiological Society HOWARD B LEWIS and H A MATTILL, The Biochemical Society V L HENDERSON and E M K GEILING, The Pharmacological Society D R HOOKER, *Secretary*

#### Baltimore, March 30-April 2, 1938

WILLIAM T PORTER, *Honorary President*, WALTER E GARREY, *Chairman*, and ANDREW C IVY, The Physiological Society GLENN E CULLEN and H A MATTILL, The Biochemical Society ARTHUR L TATUM and G PHILIP GRABFIELD, The Pharmacological Society C PHILLIP MILLER and PAUL R CANNON, The Pathological Society D R HOOKER, *Secretary*

#### Toronto, April 26-29, 1939

GLENN E CULLEN, *Chairman*, and CHARLES G KING, The Biochemical Society ARTHUR L TATUM and G PHILIP GRABFIELD, The Pharmacological Society C PHILLIP MILLER and PAUL R CANNON, The Pathological Society WALTER E GARREY and ANDREW C IVY, The Physiological Society D R HOOKER, *Secretary*

#### New Orleans, March 13-16, 1940

E M K GEILING, *Chairman*, and G PHILIP GRABFIELD, The Pharmacological Society ERNEST W GOODPASTURE and PAUL R CANNON, The Pathological Society ANDREW C IVY and PHILIP BARD, The Physiological Society WILLIAM C ROSE and CHARLES G KING, The Biochemical Society D R HOOKER, *Secretary*

#### Chicago, April 15-19, 1941

SHIELDS WARREN, *Chairman*, and H P SMITH, The Pathological Society THORNL M CARPENTER and L A MAYNARD, The Institute of Nutrition ANDREW C IVY and PHILIP BARD, The Physiological Society WILLIAM C ROSE and CHARLES G KING, The Biochemical Society E M K GEILING and G PHILIP GRABFIELD, The Pharmacological Society D R HOOKER, *Secretary*

#### Boston, March 31, April 1, 2, 3, 4, 1942

ALBERT G HOGAN, *Chairman*, and ARTHUR H SMITH, The Institute of Nutrition PHILIP BARD

and CARL J WIGGLERS, The Physiological Society RUDOLPH J ANDERSON and ARNOLD K BALLS, The Biochemical Society E M K GEILING and R N BIRTIR, The Pharmacological Society JESSIE L BOITMAN and H P SMITH, The Pathological Society SHIELDS WARREN, *Ex Chairman* D R HOOKER, *Secretary*

1943, 1944, 1945 The meetings scheduled for Cleveland were cancelled because of war conditions

PHILIP BARD, *Chairman*, and WALLACE O FENN, The Physiological Society L A DOISI and ARNOLD K BALLS, The Biochemical Society E K MARSHALL, JR and RAYMOND N BIETER, The Pharmacological Society BALDUIN LUCKE and H P SMITH, The Pathological Society LEONARD A MAYNARD and ARTHUR H SMITH, The Institute of Nutrition JACQUES J BROUENBRENNER and ARTHUR F COCA, The Association of Immunologists D R HOOKER, *Secretary*

#### Atlantic City, Mar 11, 12, 13, 14, 15, 1946

PHILIP BARD, *Chairman*, WALLACE O FENN, The Physiological Society A BAIRD HASTINGS and ARNOLD K BALLS, The Biochemical Society ERWIN L NELSON and RAYMOND N BIETER, The Pharmacological Society BALDUIN LUCKE and H P SMITH, The Pathological Society WILLIAM C ROSE and H E CARTER, The Institute of Nutrition JACQUES J BROUENBRENNER and ARTHUR F COCA, The Association of Immunologists D R HOOKER, *Secretary*

#### Chicago, May 18-22, 1947

A BAIRD HASTINGS, *Chairman*, and OTTO A BESSLEY, The Biochemical Society MAURICE H SLEIVERS and HARVEY B HAAC, The Pharmacological Society PAUL R CANNON and FRIEDA S ROBSCHT-ROBBINS, The Pathological Society ARTHUR H SMITH and H E CARTER, The Institute of Nutrition MICHAEL HLIDELBERGER and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society WILLIAM H CHAMBERS, *Secretary*

#### Atlantic City, March 15-19, 1948

MAURICE H SEEVERS, *Chairman*, and HARVEY B HAAC, *Secretary*, The Pharmacological Society DOUGLAS H SPRUNT and FRIEDA S ROBSCHT-ROBBINS, The Pathological Society R M BETHKE and H E CARTER, The Institute of Nutrition LLOYD D FELTON and ARTHUR F COCA, The Association of Immunologists WALLACE O FENN and MAURICE B VISSCHER, The Physiological Society HANS T CLARKE and OTTO A BESSLEY, The Biochemical Society WILLIAM H CHAMBERS, *Secretary*

## FEDERATION BY-LAWS

### BY-LAWS

*Adopted at the Washington Meeting, 1936 and amended at the Boston Meeting, 1942*

1 The Presidents and Secretaries of the Constituent Societies, the Chairman of the Executive Committee of the preceding year and the Federation Secretary shall form the Executive Committee of the Federation

2 The Chairmanship of the Executive Committee shall be held in turn by the Presidents of the Constituent Societies, who shall succeed one another annually in the order of seniority of the Societies

3 The Executive Committee shall appoint annually from the membership of the Federation a secretary-treasurer, to be known as the Federation Secretary

4 The Federation Secretary shall (a) Keep the minutes of the Executive Committee and distribute copies to the Secretaries of the Constituent Societies (b) Make arrangements for the Annual Meeting with the Local Committee, with the approval of the Executive Committee (c) Print in convenient combined form and distribute to the membership of the Federation the programs of the Constituent Societies as received from their respective Secretaries (d) Undertake such other duties to be decided upon from time to time by the Executive Committee, as do not conflict with the complete autonomy of the Constituent Societies

5 The Executive Committee shall control all monies in the hands of the Federation Secretary, who shall make an annual report to the Executive Committee for audit and approval The expenses of the Federation Secretary, as authorized by the Executive Committee, shall be the first charge on such monies and if insufficient for the purpose the Executive Committee shall prorate such expenses to the Constituent Societies of the Federation in proportion to their respective memberships

The Executive Committee may appropriate Federation monies annually for the uses of Local Committees and for the uses of other authorized Committees but in the latter cases an audit of expenditures shall be made and approved before such committees are discharged

6 The Executive Committee shall determine the place of the Annual Meeting, and the time shall be determined by the Local Committee, preferably within the period of March fifteenth to May first

7 The local Committee at the place of meeting of the Federation shall charge such fee for registration as may be approved by the Executive Committee The monies thus collected shall be used to defray the expenses of the Local Committee and

the remainder, after such expenses have been met, shall be turned over to the Federation Secretary

8 The Executive Committee shall consider measures of advantage to the Federation as a whole Any Constituent Society may refer similar measures to the Executive Committee No action, however, shall be taken by the Executive Committee unless specifically authorized by all the Constituent Societies

9 The Chairman of the Executive Committee may appoint committees when the purposes of such committees have been approved by all the Constituent Societies of the Federation Such committees shall be appointed for a term of one year, but may be continued and their members reappointed Such committees shall report in writing to the Executive Committee, which shall in turn report thereon to the Constituent Societies either for information or recommendation The Secretaries of the Constituent Societies shall report the recommendations of their respective Societies to the Executive Committee for final action

10 All individuals whose names appear on the program by invitation or introduction and those registering from any recognized biological laboratory may be enrolled as Associate Members of the Federation for that Annual Meeting Such Associate Members may enjoy all the privileges of the Annual Meeting except that of voting

11 No person may present orally more than one paper during all of the scientific sessions of the Constituent Societies at the time of the Annual Meeting except upon invitation of the Executive Committee or a Council Papers must be submitted to the Secretary of the Society of which the proposer is a member The proposer may request transfer to another program, but this may only be done with the consent of the Secretary of the Society concerned Any Secretary who regards any paper submitted to him as better suited to the program of another Society may arrange this transfer with the Secretary of the Society concerned, if it be possible Such transfer shall be indicated on the program

12 Abstracts not to exceed two hundred and fifty words in length, of papers approved for presentation at all of the scientific sessions of all the Constituent Societies at the Annual Meeting, shall receive publication in the *Federation Proceedings*

13 A Control Committee, consisting of at least one representative of each Constituent Society as designated by the several Councils, shall have editorial control over the *Federation Proceedings* which shall be financed as required by an annual

assessment of all the members of each Constituent Society

14 The Control Committee shall have power to choose certain additional papers presented at the Annual Meetings and from other sources, including material heretofore published in the Federation Yearbook, for publication in the Federation Proceedings

### PLACEMENT SERVICE

The Federation maintains a service to act as a medium of communication between persons seeking positions for teaching or research and institutions that wish to fill vacancies in these sciences

The service does not undertake to recommend or to pass judgment upon applicants. It aims merely to serve as a clearing house for such infor-

mation as above stated and to bring into touch with one another candidates for positions and vacancies to be filled

The Placement Service is being reorganized in line with the recommendations of the Federation Committee for its study, and to utilize certain of the facilities and forms of the Office of Scientific Personnel of the National Research Council. Individuals, whether members of the Federation or not, Universities, other institutions and organizations desiring to avail themselves of the Service may receive such information as is available. By action of the Executive Committee in 1947, a registration fee of one dollar is required of each applicant for a position.

All communications should be addressed to Dr. M. O. Lee, Director, Placement Service, 2101 Constitution Ave., Washington 25, D. C.

# THE AMERICAN PHYSIOLOGICAL SOCIETY

*Founded December 30, 1887, Incorporated June 2, 1923*

## OFFICERS, 1948-1949

*President*—MAURICE B VISSCHER, University of Minnesota, Minneapolis

*President elect*—CARL J WIGGERS, Western Reserve University, Cleveland, Ohio

*Past-President*—WALLACE O FLYNN, University of Rochester School of Medicine and Dentistry, Rochester, N Y

*Council*—MAURICE B VISSCHER, CARL J WIGGERS, WALLACE O FLYNN, EUGENE M LANDIS, Harvard Medical School, Boston, Mass, W F HAMILTON, University of Georgia School of Medicine, Augusta, H C BAZETT, University of Pennsylvania School of Medicine, Philadelphia, D B DILL (Secretary-Treasurer), Army Chemical Center, Md

*Executive Secretary*—M O LEE, 2101 Constitution Ave, Washington, D C

*Board of Publication Trustees*—ANDREW C ILL, Chairman (1946-1949), R F PITTS (1948-1950), FRANK C MANN (1948-1951)

*Representative on the Division of Biology and Agriculture of the National Research Council*—FRANCIS O SCHMITT (1945-1949)

*Representative on the Division of Medical Sciences of the National Research Council*—HALLOWELL DAVIS (1947-1950)

*Representative on the Council of the American Association for the Advancement of Science*—J H BODINE, LEIGH CHADWICK

*Historian*—WALTER J MEEK

*Contributing Editor to Scientific Monthly*—JOHN FIELD II

## PAST OFFICERS

*Organization Meeting, December 30, 1887*

S WEIR MITCHELL, *President*

H N MARTIN, *Secretary*

1888 H P BOWDITCH, President, H N MARTIN, Secretary-Treasurer, J G CURTIS, H C WOOD, H SEWALL, Councilors 1889 S WEIR MITCHELL, President, H N MARTIN, Secretary-Treasurer, H P BOWDITCH, J G CURTIS, H C WOODS, Councilors 1890 S WEIR MITCHELL, President, H N MARTIN, Secretary-Treasurer, H P BOWDITCH, J G CURTIS, H H DONALDSON, Councilors 1891 H P BOWDITCH, President, H N MARTIN, Secretary-Treasurer, R H CHITTENDEN, J G CURTIS, H N DONALDSON, Councilors 1892 H P BOWDITCH, President, H N MARTIN, Secretary-Treasurer, R H CHITTENDEN, J G CURTIS, W H HOWELL, Councilors 1893 H P

BOWDITCH, President, W P LOMBARD, Secretary-Treasurer, R H CHITTENDEN, J G CURTIS, W H HOWELL, Councilors 1894 H P BOWDITCH, President, W P LOMBARD, Secretary-Treasurer, R H CHITTENDEN, W H HOWELL, J W WARREN, Councilors 1895 H P BOWDITCH, President, F S LEE, Secretary-Treasurer, R H CHITTENDEN, W H HOWELL, W P LOMBARD, Councilors 1896 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, H P BOWDITCH, W H HOWELL, J W WARREN, Councilors 1897 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, H P BOWDITCH, W H HOWELL, W P LOMBARD, Councilors 1898 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, H P BOWDITCH, W H HOWELL, W P LOMBARD, Councilors 1899 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, W H HOWELL, W P LOMBARD, W T PORTER, Councilors 1900 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, W H HOWELL, W P LOMBARD, W T PORTER, Councilors 1901 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, W H HOWELL, W P LOMBARD, W T PORTER, Councilors 1902 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, W H HOWELL, W P LOMBARD, W T PORTER, Councilors 1903 R H CHITTENDEN, President, F S LEE, Secretary-Treasurer, W H HOWELL, W P LOMBARD, W T PORTER, Councilors 1904 R H CHITTENDEN, President, W T PORTER, Secretary-Treasurer, F S LEE, W P LOMBARD, W H HOWELL, Councilors 1905 W H HOWELL, President, L B MENDEL, Secretary, W B CANNON, Treasurer, R H CHITTENDEN, S J MELTZER, Councilors 1906 W H HOWELL, President, L B MENDEL, Secretary, W B CANNON, Treasurer, A B MACALLUM, S J MELTZER, Councilors 1907 W H HOWELL, President, L B MENDEL, Secretary, W B CANNON, Treasurer, J J ABEL, G LUSK, Councilors 1908 W H HOWELL, President, R HUNT, Secretary, W B CANNON, Treasurer, J J ABEL, G LUSK, Councilors 1909 W H HOWELL, President, R HUNT, Secretary, W B CANNON, Treasurer, A J CARLSON, W P LOMBARD, Councilors 1910 W H HOWELL, President, A J CARLSON, Secretary, W B CANNON, Treasurer, J ERLANGER, F S LEE, Councilors 1911 S J MELTZER, President, A J CARLSON, Secretary, W B CANNON, Treasurer, J ERLANGER, F S LEE, Councilors 1912 S J MELTZER, President, A J CARLSON, Secretary, W B CANNON, Treasurer, J ERLANGER, F S LEE, Councilors 1913 S J

MELTZER, President, A J CARLSON, Secretary, J ERLANGER, Treasurer, W B CANNON, F S LEE, Councilors 1914 W B CANNON, President, A J CARLSON, Secretary, J ERLANGER, Treasurer, F S LEE, S J MELTZER, Councilors 1915 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1916 W B CANNON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W E GARREY, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1917 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1918 F S LEE, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, W H HOWELL, J J R MACLEOD, W J MEEK, Councilors 1919 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, Y HENDERSON, J J R MACLEOD, W J MEEK, Councilors 1920 W P LOMBARD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, W B CANNON, J J R MACLEOD, Y HENDERSON, C J WIGGERS, Councilors 1921 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, J A E EYSTER, Y HENDERSON, C J WIGGERS, A J CARLSON, Councilors 1922 J J R MACLEOD, President, C W GREENE, Secretary, J ERLANGER, Treasurer, Y HENDERSON, C J WIGGERS, A J CARLSON, Councilors 1923 A J CARLSON, President, C W GREENE, Secretary, J ERLANGER, Treasurer, C J WIGGERS, A B LUCKHARDT, J A E EYSTER, J R MURLIN, Councilors 1924 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, A B LUCKHARDT, J A E EYSTER, J R MURLIN, W E GARREY, Councilors 1925 A J CARLSON, President, W J MEEK, Secretary, C K DRINKER, Treasurer, J A E EYSTER, J R MURLIN, W E GARREY, JOSEPH ERIANCLER, Councilors 1926 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, J R MURLIN, W E GARREY, A B LUCKHARDT, C J WIGGERS, Councilors 1927 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, W E GARREY, A B LUCKHARDT, C J WIGGERS, R GESELL, Councilors 1928 J ERLANGER, President, W J MEEK, Secretary, A FORBES, Treasurer, A B LUCKHARDT, C J WIGGERS, R GESELL, A J CARLSON, Councilors 1929 W J MEEK, President, ALFRED C REDFIELD, Secretary, A FORBES, Treasurer, C J WIGGERS, R GESELL, A J CARLSON, J R MURLIN, Councilors 1930 W J MEEK, President, ARNO B LUCKHARDT, Secretary, A FORBES, Treasurer, R GESELL, A J CARLSON, J R MURLIN, E G MARTIN, Councilors 1931 W J MEEK, Presi-

dent, ARNO B LUCKHARDT, Secretary, ALEXANDER FORBES, Treasurer, A J CARLSON, J R MURLIN, E G MARTIN, JOHN TAIT, Councilors 1932 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, E G MARTIN, W J MEEK, J R MURLIN, JOHN TAIT, Councilors 1933 ARNO B LUCKHARDT, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ERNEST G MARTIN, W J MEEK, JOHN TAIT, Councilors 1934 CHARLES W GREENE, President, FRANK C MANN, Secretary, ALEXANDER FORBES, Treasurer, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, JOHN TAIT, Councilors 1935 FRANK C MANN, President, ANDREW C IVY, Secretary, ALEXANDER FORBES, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, W J MEEK, Councilors 1936 FRANK C MANN, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1937 WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, ARNO B LUCKHARDT, Councilors 1938 WILLIAM T PORTER, Honorary President, WALTER E GARREY, President, ANDREW C IVY, Secretary, WALLACE O FENN, Treasurer, ARNO B LUCKHARDT, CHARLES H BEST, PHILIP BARD, HERBERT S GASSER, Councilors 1939 ANDREW C IVY, President, PHILIP BARD, Secretary, WALLACE O FENN, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1940 ANDREW C IVY, President, PHILIP BARD, Secretary, CARL J WIGGERS, Treasurer, CHARLES H BEST, HERBERT S GASSER, ARNO B LUCKHARDT, MAURICE B VISSCHER, Councilors 1941 PHILIP BARD, President, CARL J WIGGERS, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, ARNO B LUCKHARDT, MAURICE B VISSCHER, HIRAM E ESSEX, Councilors 1942, 1943, 1944, 1945 PHILIP BARD, President, WALLACE O FENN, Secretary, HALLOWELL DAVIS, Treasurer, CHARLES H BEST, MAURICE B VISSCHER, HIRAM E ESSEX, W F HAMILTON, Councilors 1946 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, CHARLES H BEST, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors 1947 WALLACE O FENN, President, MAURICE B VISSCHER, Secretary, D B DILL, Treasurer, Eugene M LANDIS, HIRAM E ESSEX, W F HAMILTON, H C BAZETT, Councilors

## CONSTITUTION

### I

1 This Society shall be named "THE AMERICAN PHYSIOLOGICAL SOCIETY, INCORPORATED"

2 The Society is instituted to promote the advance of Physiology and to facilitate personal intercourse between American Physiologists

## II

1 The Society shall consist of members and honorary members

2 Any person who has conducted and published meritorious original researches in Physiology and who is a resident of North America shall be eligible for membership in the Society

3 Members who have been relieved by the Council of the payment of the annual assessment shall retain all the rights of members

4 Distinguished men of science who have contributed to the advance of Physiology shall be eligible for election as honorary members of the Society. Honorary members shall pay no membership fee. They shall have the right of attending the meetings of the Society, and of taking part in its scientific discussions, but they shall have no vote

## III

1 The management of the Society shall be vested in a Council consisting of a President elect, President, Past-President for the previous year, and four other members. The President elect and one member of the Council shall be chosen by ballot at each annual meeting. The President elect shall automatically assume the duties of President at the adjournment of the annual meeting following his or her election. The four additional members shall be elected for terms of four years and shall not be eligible to succeed themselves. A person who has once been President shall not be eligible for reelection as President elect. The Council shall have the power to elect its Secretary-Treasurer from among its own members. It shall also have the power to appoint and to compensate an Executive Secretary of the Society, who shall not be a voting member of the Council but shall assist it in carrying on the functions of the Society, including the receipt and disbursement of funds under the direction of the Council. If the annual meeting is not held all the members of the Council shall continue in office until their successors are chosen in the prescribed manner and succession

2 The Council shall have the power to fill all interim vacancies that may occur in its membership or in any Committee or board of the Society except those for which other provisions have been made

## IV

1 At least a fortnight before the annual meeting the Secretary shall send to each member a notice of the place and time of each meeting, and shall make such other announcements as the Council shall direct

2 The annual assessment shall be determined by the Council, and shall be due in advance at the time of the annual meeting. No allocation or disbursement of funds of the Society shall be made except upon prior approval of the Council. Appropriations shall be made by the Council for the conduct of the necessary and appropriate business of the Society

3 Any member whose assessment is two years in arrears shall cease to be a member of the Society, unless at the next annual meeting he shall be reinstated by special vote of the Society, and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to said meeting

4 Any member who has retired because of illness or age may, upon application to the Council be relieved from payment of the annual assessment

## V

1 Meetings of the Society for the conduct of business and the presentation of papers and demonstrations shall be held annually except for national emergencies or other exceptional circumstances when the Council may cancel the proposed meeting. The time and place of such meetings shall be determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology

2 Special meetings may be held at such times and places as the Council may determine

## VI

1 Proposed amendments to the Constitution must be brought up at one meeting for preliminary discussion and approval by a majority vote and cannot be adopted except by a two thirds vote at a business session at the next annual meeting. Notice of such changes shall be sent to all members at least two weeks prior to the meeting at which they are scheduled for adoption

2 At all business meetings of the Society twenty five members shall form a quorum

3 By laws for the conduct of the Society may be adopted, altered, or repealed at any business meeting by two thirds vote of the ballots cast

## VII

1 The Council may, from the names of the candidates proposed in writing by at least two members of the Society, nominate candidates for election to membership. The names of the candidates so nominated and a statement of their qualifications for membership signed by their proposers shall be available for inspection during the business sessions of the Society at which their election is considered. The candidates may be balloted for at any session of the same meeting and a majority vote shall elect. If an annual meeting is not

held, the Council shall elect the candidates to membership subject to Society approval at the next annual meeting

2 Honorary members shall be proposed by the Council, and shall be elected by a majority ballot of the members present at an annual business session of the Society

### VIII

1 If a majority of the Council shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and if two thirds of the members present vote for it, the member shall be expelled, and his assessment for the current year shall be returned to him and he shall cease to be a member of the Society

### IX

1 The official organs of the Society shall be the American Journal of Physiology, the Physiological Reviews and such other publications as the Society shall establish. These the Society shall own and they shall be managed according to the provisions of Article X

### X

1 The President of the Society shall appoint, in consultation with the Council and subject to the approval of the Society, three members of the Society to serve as members of a Board of Publication Trustees

2 The initial appointments shall be for one, two and three years. Thereafter, each member shall be appointed for three years, and shall be eligible for one immediate reappointment. He may be subsequently reappointed, but only after the lapse of at least one year between reappointments

3 The Board of Publication Trustees shall be

vested with full power of the Society to control and manage, both editorially and financially, all of the publications owned in whole or in part by the Society, to appoint editorial boards, to appoint and compensate a Managing Editor, and to control all publication funds, none of which, however, may be diverted from support of publications of the Society except by consent of the Council

4 The Board of Publication Trustees shall make a full report to the Council at each annual meeting of the financial condition and publication policy of the Journals or other publications

### BY-LAWS

1 All papers read before the Society shall be limited to a length of ten minutes. No person may orally present more than one paper. In case of joint authorship the name of the individual who will orally present the paper shall stand first

2 Abstracts in duplicate, not to exceed two hundred and fifty words in length, of all papers to be presented at the annual meeting of the Society shall be required by the Secretary for publication in the Federation Proceedings, in accordance with rules approved by the Council

3 The Council shall upon the request of twenty-five members call a regional meeting of the Society at any time and place, for the reading of papers and the promotion of personal intercourse. Such a request shall be made in writing at least six weeks before the proposed date of meeting. Such meeting shall be held in accordance with the Constitution and By-laws of the Society, and if the regular officers of the Society cannot be present the President shall appoint a committee from among the petitioners to conduct the meeting. The Committee through a Secretary chosen by them shall forward an account of the scientific proceedings of the meeting to the official Secretary of the Society for insertion in the minutes

4 No general business of the Society shall be transacted at such regional meetings



# AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED

*Founded December 6, 1906, Incorporated September 12, 1919*

## OFFICERS, COMMITTEES AND REPRESENTATIVES FOR 1948-1949

*President*—HANS T CLARKE, College of Physicians and Surgeons, Columbia University, New York, N Y

*Vice-President*—CARL F CORI, Washington University School of Medicine, St Louis, Mo

*Secretary*—OTTO A BESSEY, University of Illinois College of Medicine, Chicago, Ill

*Treasurer*—E A EVANS, JR, University of Chicago, Chicago, Ill

*Council*—ARNOLD KENT BALLS, OTTO A BESSEY, CARL F CORI, HANS T CLARKE, E A EVANS, JR, A BAIRD HASTINGS, J MURRAY LUCK

*Editorial Committee*—HENRY A MATTILL, *Chairman*, W R BLOOR, HENRY D DAKIN, JOHN T EDSALL, C G KING, J MURRAY LUCK, ALFRED N RICHARDS, PHILIP A SHAFFER, DAVID WRIGHT WILSON

*Editorial Board*—RUDOLPH J ANDERSON, *Managing Editor*, R M ARCHIBALD, W MANSFIELD CLARK, HANS T CLARKE, CARL F CORI, E A DOISY, V DU VIGNEAUD, JOSEPH S FRUTON, A BAIRD HASTINGS, HOWARD B LEWIS, ELMER V MCCOLLUM, WILLIAM C ROSE, WILLIAM C STADIE, EDWARD L TATUM, DONALD D VAN SLYKE, HUBERT B VICKERY

*Nominating Committee*—WILLIAM C ROSE, *Chairman and Secretary*, ERIC G BALL, C A ELVEHJEM, JOSEPH S FRUTON, C G KING, SEVERO OCHOA, W M STANLEY, JAMES B SUMNER, HUBERT B VICKERY

*Committee on Biochemical Nomenclature* (Jointly with the American Institute of Nutrition)—C A ELVEHJEM, *Chairman*, E M NELSON

*Committee on Clinical Chemistry*—HANS T CLARKE, ARNOLD C OSTERBERG, WARREN M SPERRY, DONALD D VAN SLYKE

*Finance Committee*—HANS T CLARKE, *Chairman*, RUDOLPH J ANDERSON, E A EVANS, JR, DONALD B VAN SLYKE

*Committee on Professional Training of Biochemists*—HOWARD B LEWIS, *Chairman*, W MANSFIELD CLARK, E A DOISY, V DU VIGNEAUD, C A ELVEHJEM

*Committee on Relations of Biochemists to the Army Medical Service Corps*—WENDELL H GRIFFITH, *Chairman*, DOUGLAS A MACFADYEN, CLIVE M McCAY

*Representatives to the Council of the American Association for the Advancement of Science*—W D ARMSTRONG, DEAN BURK

*Representative to the American Documentation Institute*—ATHERTON SEIDELL

*Representatives to the National Research Council*—Division of Biology and Agriculture, CLIVE M McCAY, Division of Medical Sciences, WILLIAM C STADIE

*Historian*—PHILIP A SHAFFER

## PAST OFFICERS

1907 RUSSELL H CHITTENDEN, President, J J ABEL, Vice-President, W J GIES, Secretary, L B MENDEL, Treasurer, W JONES, W KOCH, J MARSHALL, T B OSBORNE, Councilors 1908 JOHN J ABEL, President, OTTO FOLIN, Vice-President, WM J GIES, Secretary, L B MENDEL, Treasurer, A B MACALLUM, A P MATHEWS, F G NOVY, Councilors 1909 OTTO FOLIN, President, T B OSBORNE, Vice President, WM J GIES, Secretary, L B MENDEL, Treasurer, J J ABEL, P A LEVENE, G LUSK, Councilors 1910 THOMAS B OSBORNE, President, L B MENDEL, Vice-President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, A B MACALLUM, A P MATHEWS, V C VAUGHAN, Councilors 1911 LAFAYETTE B MENDEL, President, A B MACALLUM, Vice President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, WM J GIES, A S LOEVENHART, P A SHAFFER, Councilors 1912 ARCHIBALD B MACALLUM, President, G LUSK, Vice-President, A N RICHARDS, Secretary, WALTER JONES, Treasurer, H P ARMSBY, L B MENDEL, H G WELLS, Councilors 1913 ARCHIBALD B MACALLUM, President, G LUSK, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, H P ARMSBY, L B MENDEL, H G WELLS, Councilors 1914 GRAHAM LUSK, President, C L ALSBERG, Vice President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, J J ABEL, A B MACALLUM, T B OSBORNE, Councilors 1915 WALTER JONES, President, C L ALSBERG, Vice-President, P A SHAFFER, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, G LUSK, L B MENDEL, Councilors 1916 WALTER JONES, President, F P UNDERHILL, Vice President, S R BENEDICT, Secretary, D D VAN SLYKE, Treasurer, OTTO FOLIN, A B MACALLUM, P A SHAFFER, Councilors 1917 CARL L

ALSBERG, President, A P MATHEWS, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, L J HENDERSON, P A SHAFFER, F P UNDERHILL, Councilors 1918 CARL L ALSBERG, President, A P MATHEWS, Vice-President, S R BENEDICT, Secretary, H C BRADLEY, Treasurer, W J GIES, ANDREW HUNTER, E V MCCOLLUM, Councilors 1919 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, ANDREW HUNTER, E V MCCOLLUM, L B MENDEL, Councilors 1920 STANLEY R BENEDICT, President, D D VAN SLYKE, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, OTTO FOLIN, WALTER JONES, L B MENDEL, Councilors 1921 DONALD D VAN SLYKE, President, P A SHAFFER, Vice-President, V C MYERS, Secretary, H C BRADLEY, Treasurer, S R BENEDICT, OTTO FOLIN, WALTER JONES, Councilors 1922 DONALD D VAN SLYKE, President, P A SHAFFER, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, S R BENEDICT, H C BRADLEY, A P MATHEWS, Councilors 1923 PHILIP A SHAFFER, President, H C SHERMAN, Vice-President, V C MYERS, Secretary, W R BLOOR, Treasurer, H C BRADLEY, ANDREW HUNTER, A P MATHEWS, Councilors 1924 PHILIP A SHAFFER, President, HENRY C SHERMAN, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, ANDREW HUNTER, VICTOR C MYERS, Councilors 1925 HENRY C SHERMAN, President, EDWARD C KENDALL, Vice-President, D WRIGHT WILSON, Secretary, WALTER R BLOOR, Treasurer, OTTO FOLIN, LAFAYETTE B MENDEL, PHILIP A SHAFFER, Councilors 1926 EDWARD C KENDALL, President, ELMER V MCCOLLUM, Vice-President, FRED C KOCH, Secretary, GLENN E CULLEN, Treasurer, J B COLLIP, EDWARD A DOISY, ALBERT P MATHEWS, Councilors 1927 E V MCCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, E A DOISY, F C KOCH, D C VAN SLYKE, Councilors 1928 E V MCCOLLUM, President, W R BLOOR, Vice-President, D WRIGHT WILSON, Secretary, G E CULLEN, Treasurer, WM M CLARK, F C KOCH, D D VAN SLYKE, Councilors 1929 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, C L A SCHMIDT, P A SHAFFER, Councilors 1930 W R BLOOR, President, H C BRADLEY, Vice-President, H B LEWIS, Secretary, G E CULLEN, Treasurer, W M CLARK, P A SHAFFER, D W WILSON, Councilors 1931 H C BRADLEY, President, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, W C ROSE, P A SHAFFER, D W WILSON, Councilors 1932 H C BRADLEY, Presi-

dent, W M CLARK, Vice-President, H B LEWIS, Secretary, C H FISKE, Treasurer, P E HOWE, W C ROSE, D W WILSON, Councilors 1933 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, P E HOWE, W C ROSE, Councilors 1934 W M CLARK, President, H B LEWIS, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, E A DOISY, P E HOWE, Councilors 1935 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, C H FISKE, Treasurer, H C BRADLEY, J B COLLIP, E A DOISY, Councilors 1936 H B LEWIS, President, G E CULLEN, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, J B COLLIP, E A DOISY, W C ROSE, Councilors 1937 G E CULLEN, President, W C ROSE, Vice-President, H A MATTILL, Secretary, A B HASTINGS, Treasurer, E A DOISY, H B LEWIS, H B VICKERY, Councilors 1938 G E CULLEN, President, W C ROSE, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, H B VICKERY, Councilors 1939 W C ROSE, President, R J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H B LEWIS, H A MATTILL, G E CULLEN, Councilors 1940 WILLIAM C ROSE, President, RUDOLPH J ANDERSON, Vice-President, CHARLES G KING, Secretary, A B HASTINGS, Treasurer, H A MATTILL, GLENN E CULLEN, E A DOISY, Councilors 1941 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, H B LEWIS, W C ROSE, Councilors 1942 R J ANDERSON, President, E A DOISY, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, C A KING, H Y CLARKE, Councilors 1943 E A DOISY, President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, W C ROSE, H T CLARKE, R J ANDERSON, Councilors 1944 E A DOISY, President, A B HASTINGS, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, H T CLARKE, V DU VIGNEAUD, Councilors 1945 A B HASTINGS, President, H T CLARKE, Vice-President, A K BALLS, Secretary, W C STADIE, Treasurer, R J ANDERSON, C F CORI, V DU VIGNEAUD, Councilors 1946 A B HASTINGS, President, H T CLARKE, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, JR, Treasurer, V DU VIGNEAUD, C F CORI, A K BALLS, Councilors 1947 HANS T CLARKE, President, CARL F CORI, Vice-President, OTTO A BESSEY, Secretary, E A EVANS, JR, Treasurer, A K BALLS, A BAIRD HASTINGS, J MURRAY LUCK, Councilors

## CONSTITUTION

## FROM THE ARTICLES OF INCORPORATION

1 The name of the proposed corporation is "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED"

2 The purposes for which this corporation is formed are to further the extension of biochemical knowledge and to facilitate personal intercourse between American investigators in biological chemistry

## BY-LAWS

ARTICLE I—*Membership*

SECTION 1 *Eligibility for Membership*—Qualified investigators who have conducted and published meritorious original investigations in biological chemistry shall be eligible for membership in the Society

SEC 2 *Nomination*—Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

SEC 3 *Election to Membership*—A A nominee for membership may be voted for by ballot at any meeting of the Society after Council has reported its findings on his eligibility. The eligible candidate shall be reported by the Council as "eligible" or as "eligible and indorsed." B A majority of the ballots cast shall elect

SEC 4 *Forfeiture*—A Any member who may grant the use of his name for (a) the advertisement of a patent medicine, a proprietary food preparation, or any other commercial article of doubtful value to the public or possibly harmful to the public health, or (b) who may concede its use for the purpose of encouraging the sale of individual samples (of any such product) that he has not examined, shall forfeit his membership

B The Council shall have authority to announce forfeiture of membership, provided that the copy of the charges, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice, shall have been delivered to the member charged with violating the preceding section either personally or mailed to him at his last known address at least thirty days before the date of such hearing

SEC 5 *Expulsion*—Upon the recommendation of the Council any member may be expelled by a majority vote of the total membership at a meeting of the Society, provided that a copy of the charges against him, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice shall have been delivered to him personally or mailed to him at his

last known address at least thirty days before the date of such hearing

ARTICLE II—*Meetings and Quorum*

SECTION 1 *Annual*—The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

SEC 2 *Special*—A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice President, and must be called at the request of a majority of the Council or fifteen members of the Society. A notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held

SEC 3 *Quorum*—Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date

ARTICLE III—*Officials*

SECTION 1 *Officers*—The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society

SEC 2 *Council*—A The officers so elected and three additional members, one of whom shall be elected at each annual meeting of the Society to serve a three year term, shall constitute the Board of Directors of the corporation and shall be known as "The Council" (When this provision is first put into effect three members will need to be elected for a one, a two and a three year period.)

B No two members of the Council may be from the same institution, and none of the officers so elected shall be eligible for re election for more than two years except the Secretary and Treasurer, who shall be eligible for re election for five years. The three additional members of the Council shall be ineligible for re election (until after the lapse of one year)

SEC 3 *Duties of Officers*—The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

SEC 4 *Assistant Treasurer*—A The Council may from time to time appoint a trust company, or some member of the Society, to serve during the pleasure of the Council as Assistant Treasurer, and to act as depository of the investments and income of the "Christian A Herter Memorial Fund" and of such other funds as the Society may from time to time commit to its or his charge

B The Assistant Treasurer shall have and exercise the following powers and duties, viz, the custody and safe keeping of securities and cash belonging to the "Christian A Herter Fund" and the collection of income and other moneys due to

# AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED

*Founded December 28, 1908, Incorporated June 19, 1933*

## OFFICERS, 1948-1949

*President*—CARL A DRAGSTEDT, Northwestern University Medical School, Chicago, Ill

*Vice-President*—H B VAN DYKE, Columbia University, College of Physicians and Surgeons, New York City

*Secretary*—HARVEY B HAAG, Medical College of Virginia, Richmond, Va

*Treasurer*—K K CHEN, Lilly Research Laboratories, Indianapolis, Ind

*Council*—ARTHUR C DEGRAFF, New York University College of Medicine, New York City, ROBERT A WOODBURY, University of Tennessee, Memphis, GORDON A ALLS, California Institute of Technology, Pasadena CARL A DRAGSTEDT, H B VAN DYKE, HARVEY B HAAG, K K CHEN

*Membership Committee*—CHARLES M GRUBER (term expires 1949), Jefferson Medical College, Philadelphia, Pa, ROBERT P WALTON (term expires 1950), Medical College of the State of South Carolina, Charleston, JOHN C KRANTZ, JR, (term expires 1951) University of Maryland Medical School, Baltimore

*Nominating Committee*—BENJAMIN H ROBBINS, Vanderbilt University School of Medicine, Nashville, Tenn, C JELLEFF CARR, University of Maryland School of Medicine, Baltimore, WILLIAM T SALTER, Yale University School of Medicine, New Haven, Conn, ALFRED GILMAN, Columbia University College of Physicians and Surgeons, New York City, OTTO KRAYLER, Harvard University Medical School, Boston, Mass

*Historian*—WILLIAM DEB MACNIDER, University of North Carolina, Chapel Hill

*Committee on International Pharmacological Congress*—M L TAINTER, Chairman, Sterling-Winthrop Research Institute, Rensselaer, N Y, OTTO KRAYLER, Harvard University Medical School Boston, Mass, H B VAN DYKE, Columbia University College of Physicians and Surgeons, New York City

## PAST OFFICERS

1909 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, S J MELTZER, T SOLLMANN, C W EDMUNDS, A C CRAWFORD, Councilors 1910 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, A C CRAWFORD, G B WALLACE, Councilors 1911 J J ABEL, President, REID HUNT, Secretary, A S LOEVENHART, Treasurer, G B WALLACE, W DEB MACNIDER, Councilors, 1912 J J ABEL, President, J AUER, Secretary, A S LOEVENHART, Treasurer, G B WALLACE,

REID HUNT, Councilors 1913 T SOLLMANN, President, J AUER, Secretary, A S LOEVENHART, Treasurer, J J ABEL, W DEB MACNIDER, Councilors 1914 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, J J ABEL, A S LOEVENHART, Councilors 1915 T SOLLMANN, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, WORTH HALE, D E JACKSON, Councilors 1916 REID HUNT, President, J AUER, Secretary, W DEB MACNIDER, Treasurer, A D HIRSCHFELDER, G B ROTH, Councilors 1917 REID HUNT, President, L G ROWNTREE, Secretary, W DEB MACNIDER, Treasurer, J AUER, CARL VOEGTLIN, Councilors 1918 REID HUNT, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, HUGH MCGUIGAN, CARL VOEGTLIN, Councilors 1919 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, REID HUNT, E K MARSHALL, JR, Councilors 1920 A S LOEVENHART, President, E D BROWN, Secretary, W DEB MACNIDER, Treasurer, D E JACKSON, E K MARSHALL, JR, Councilors 1921 C W EDMUNDS, President, E D BROWN, Secretary, HUGH MCGUIGAN, Treasurer, JOHN AUER, J P HANZLIK, Councilors 1922 C W EDMUNDS, President, E D BROWN, Secretary, HUGH MCGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1923 C W EDMUNDS, President, E D BROWN, Secretary, HUGH MCGUIGAN, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1924 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, J P HANZLIK, H G BARBOUR, Councilors 1925 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1926 JOHN AUER, President, E D BROWN, Secretary, A L TATUM, Treasurer, H G BARBOUR, W DEB MACNIDER, Councilors 1927 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1928 CARL VOEGTLIN, President, E D BROWN, Secretary, A L TATUM, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1929 CARL VOEGTLIN, President, E D BROWN, Secretary, O H PLANT, Treasurer, V E HENDERSON, C W EDMUNDS, Councilors 1930 GEORGE B WALLACE, President, E D BROWN, Secretary, O H PLANT, Treasurer, H G BARBOUR, C M GRUBER, Councilors 1931 GEORGE B WALLACE, President, VELIEN E HENDERSON, Secretary, O H PLANT, Treasurer, PAUL D LAMSON, WILLIAM DEB MACNIDER, Councilors 1932 WM DEB MACNIDER, Presi-

dent, A N RICHARDS, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, G B ROTH, A L TATUM, Councilors 1933 WM DEB MACNIDER, President, A L TATUM, Vice-President, V E HENDERSON, Secretary, O H PLANT, Treasurer, C M GRUBER, G B ROTH, Councilors 1934 R A HATCHER, President, A L TATUM, Vice-President, E M K GEILING, Secretary, O H PLANT, Treasurer, WM DEB MACNIDER, R L STEHLE, Councilors 1935 V E HENDERSON, President, O H PLANT, Vice-President, E M K GEILING, Secretary, C M GRUBER, Treasurer, FLOYD DE EDS, M S DOOLEY, Councilors 1936 V E HENDERSON, President, O H PLANT, Vice-President, E M K GEILING, Secretary, C M GRUBER, Treasurer, C W EDMUNDS, G B WALLACE, Councilors 1937 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, V E HENDERSON, M H SEEVERS, Councilors 1938 A L TATUM, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, C M GRUBER, Treasurer, E K MARSHALL, JR, C F SCHMIDT, Councilors 1939 O H PLANT, President, E M K GEILING, Vice-President, G P GRABFIELD, Secretary, E E NELSON, Treasurer, A L TATUM, C A DRAGSTEDT, Councilors 1940 E M K GEILING, President, C F SCHMIDT, Vice-President, G PHILIP GRABFIELD, Secretary, E E NELSON, Treasurer, B H ROBBINS, C H THIENES, Councilors 1941 E M K GEILING, President, C F SCHMIDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, E G GROSS, R G SMITH, Councilors 1942 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, McK CATTELL, R G SMITH, Councilors 1943 E K MARSHALL, JR, President, CARL A DRAGSTEDT, Vice-President, RAYMOND N BIETER, Secretary, E E NELSON, Treasurer, McK CATTELL, R G SMITH, Councilors 1944, 1945 E E NELSON, President, C M GRUBER, Vice President, R N BIETER, Secretary, McKEEN CATTELL, Treasurer, HARRY BECKMAN, NATHAN B EDDY, Councilors 1946 MAURICE H SLEEVES, President, H B VAN DYKE, Vice-President, HARVEY B HAAG, Secretary, McKEEN CATTELL, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors 1947 MAURICE H SLEEVES, President, CARL A DRAGSTEDT, Vice President, HARVEY B HAAG, Secretary, K K CHEN, Treasurer, HAMILTON H ANDERSON, JOHN C KRANTZ, JR, Councilors

## CONSTITUTION

### ARTICLE I—Name

The name of this organization shall be the "AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED"

### ARTICLE II—Objects

The purpose of this Society shall be to promote these branches of science and to facilitate personal intercourse between investigators who are actively engaged in research in these fields

### ARTICLE III—Membership

SECTION 1 Any person who has conducted and published a meritorious investigation in pharmacology or experimental therapeutics, and who is an active investigator in one of these fields, shall be eligible to membership, subject to the conditions of the other sections of Article III

SEC 2 A Candidates for membership to this Society shall be proposed by two members who are not members of the Council. The names so proposed shall be sent to the Secretary at least three months prior to the Annual Meeting

B The Membership Committee shall investigate the qualifications of the candidates and report to the Council

C Candidates reported upon by the Membership Committee to the Council may be recommended for admission by the Council only provided they have been approved by four-fifths of the combined membership of the Membership Committee and the Council

D The names of the candidates recommended for admission by the Council shall be posted by the Secretary not later than the day preceding the election for members

E The election of members shall be by individual ballot, one opposing vote in every eight cast shall be sufficient to exclude a candidate from membership

### SEC 3 Forfeiture of Membership

A Any member whose assessment is three years in arrears shall cease to be a member of the Society, unless he shall be reinstated by a special vote of the Council, and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to the Council

B If the Council shall decide that it is for the best interests of the Society that a member be expelled, the member shall be notified and given an opportunity of a hearing before the Council. Upon the recommendation of the Council the member then may be expelled by a three fourths vote of those present at a regular meeting of the Society

### SEC 4 Honorary Members

A Distinguished men of science who have contributed to the advance of pharmacology or experimental therapeutics shall be eligible for election as honorary members of the Society

B Nominations for honorary members shall take the same course as nominations for ordinary members (Art III, Sec 2), but their election shall

require the unanimous vote of the members present at the election

C Honorary members shall pay no membership fee. They shall have the right to attend all meetings of the Society, and to take part in its discussions, but they shall have no vote.

D The conditions for continuation of membership shall be the same for honorary as for ordinary members (Art. III, Sec. 3), except that forfeiture for arrears of fees does not apply to honorary members.

#### ARTICLE IV —*Officers and Elections*

##### SECTION 1 *Officers and Committees*

A The management of the Society shall be vested in a Council of seven officers, consisting of the President, Vice-President, Secretary, and Treasurer of the Society, and three Councilors-at-Large.

B The four ex officio members shall serve for one year but shall be eligible for re-election.

C The three Councilors-at-Large shall serve for a period of three years, and shall not be eligible for immediate re-election.

D At the first meeting of the Society under this amended Constitution one Councilor at-Large shall be elected to serve for a period of three years, one for two years, and one for one year, and subsequently one Councilor at-Large shall be elected annually to serve for a period of three years.

E There shall be a Membership Committee, consisting of three members. No two members shall be from the same institution. The election of the Membership Committee shall be held annually at the time when the election of officers occurs. At the first meeting of the Society under this constitution, one member shall be elected to serve on the Committee for three years, one for two years, and one for one year, and subsequently one member shall be elected each year to serve for a period of three years.

F There shall be a Nominating Committee of five members. No two members shall be from the same institution. Members of the Nominating Committee shall serve for one year. They are eligible for re-election, but shall not hold membership in the Committee for more than two consecutive years.

##### SEC. 2 —*Nomination of Officials and Committees*

A The Nominating Committee shall make at least one nomination for each office and for the position on the Membership Committee to be filled by vote of the members. The nominations so made shall be transmitted to the Secretary and by him in turn to the members, at least one month before the annual meeting.

B Nominations for membership on the Nominating Committee shall be made by individual members at the time of the annual election. The

five nominees who receive the highest numbers of votes shall be declared elected. The Nominating Committee shall select its own Chairman who shall serve as Secretary to the Committee.

##### SEC. 3 *Election of Officials and Committeemen*

A At the opening of the first executive session of the annual meeting the Secretary shall give to each member present a printed ballot showing the nominations of the Nominating Committee. After accepting additional nominations from individual members present a complete list of nominees shall be posted. A preliminary vote shall then be taken and the tellers, appointed by the President to conduct the election, shall post immediately a final list showing the two nominees for each office receiving the highest number of votes. At the close of the first session, a final vote shall be taken. A majority of votes cast shall be necessary to a choice.

B Such vacancies as may occur in the offices and in the various committees in the interval between annual meetings shall be filled by a majority vote of the Council.

#### ARTICLE V —*Meetings*

SECTION 1 The annual meeting of the Society shall be held at a time and place determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology.

SEC. 2 Special meetings may be held at such times and places as the Council may determine.

SEC. 3 At least four weeks before the annual meeting the Secretary shall send to each member a notice of the time and place of such meeting and shall make such announcements as the Council may direct.

#### ARTICLE VI —*Financial*

SECTION 1 The annual assessment shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due in advance at the time of the meeting.

SEC. 2 Beyond the ordinary expenditures required by the routine business of the Society no money shall be disbursed save by the authority of the Council or Society.

SEC. 3 The treasurer shall make an annual report to the Society.

SEC. 4 In case any profits result to the Society from the Journal of Pharmacology and Experimental Therapeutics at the end of the financial year, such profits shall be kept in a special account, after deducting any sums expended by the Society during the year for the conduct of the Journal, and shall be held subject to the order of the Council on recommendation of the Editorial Board.

#### ARTICLE VII —*Quorum*

Ten members shall constitute a quorum for the transaction of business.

ARTICLE VIII —*By-Laws*

By-Laws shall be adopted, altered or repealed at any meeting by two thirds vote of the ballots cast

ARTICLE IX —*Amendments*

SECTION 1 Intended amendments to the Constitution shall be sent to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be indorsed in writing by at least three members

SEC 2 The Secretary shall give all members due notice of proposed amendments

SEC 3 A four-fifths vote of the members present shall be required for the adoption of an amendment

ARTICLE X —*Official Publications*

SECTION 1 The President of the Society shall appoint, in consultation with the Council, and subject to the approval of the Society, three members of the Society to serve as members of a Board of Publications Trustees, these members shall elect a Managing Editor for each of the official journals of the Society, and each Managing Editor, during the term of his service, shall act as an additional voting member of the Board of Publications Trustees and shall participate in all the activities of the Board except that of election of managing editors of the journals of the Society

SEC 2 The members of the Board of Publications Trustees, hereinafter termed "The Board," shall each serve for a term of three years, shall be subject to reappointment, and may hold office concurrently in the Society. At the first appointment, however, one member shall be appointed for three years, one member for two years, and one member for one year, in order that in the future, appointments may be made annually, in rotation. The Board shall meet at least once annually (a quorum shall consist of three members) and shall report directly to the Society

SEC 3 The special functions of The Board shall be to consider and to investigate thoroughly all matters pertaining to the fiscal and editorial policies of the journals which may come to the Society or to its Council, to the Managing Editors, and to the members of The Board. The Board shall (1)

administer the finances of the journals, (2) establish the publication policies of the journals, and (3) elect a Managing Editor for each of the journals of the Society (as described in Sec 1)

SEC 4 The Managing Editor of each journal shall nominate to The Board, members of the Society acceptable to him as Associate Editors. From those nominated, The Board shall elect, for each journal, Associate Editors in such number as they shall consider adequate to fulfill the duties of that Editorial Board. The Managing Editor and each Associate Editor shall serve for three years, subject to reappointment, and may hold office concurrently in the Society. In the choice of The Boards of Editors, The Board is charged with the responsibility of obtaining editors with expert knowledge in the several fields of pharmacological research and with evidenced ability for critical and grammatical expression

SEC 5 The Boards of Editors of the journals shall meet on call of the respective Managing Editor, if possible just prior to or during the regular meetings of the Society, and may make recommendations to The Board concerning the improvement of the publication policies of the journals

## BY-LAWS

1 Papers to be read shall be submitted by the members of the Society to the Secretary, who, with the President, shall be empowered to arrange the program. No person may orally present more than one paper. In case of joint authorship, the name of the individual who will orally present the paper shall stand first. Papers not read shall appear on the program as read by title

2 An abstract of a paper to be read before the Society shall be sent to the Secretary with the title. As early as possible after each meeting, the Secretary shall edit and publish the Proceedings of the Society together with abstracts in a publication authorized by the Society

3 All applications for membership shall be accompanied by a copy of as many reprints as possible of the published work of the applicant

4 Any member who has been an active member for thirty years, or who has retired for disability or age may, upon notification to the Treasurer be relieved from payment of dues

# THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

*Founded December 29, 1913*

## OFFICERS, 1948-1949

*President*—H P SMITH, Columbia University College of Physicians and Surgeons, New York City

*Vice-President*—JOHN G KIDD, Cornell University School of Medicine, New York City

*Secretary-Treasurer*—FRIEDA S ROBSCHILT-ROBBINS, University of Rochester School of Medicine, Rochester, N Y

*Councilors*—JAMES F RINEHART, University of California Medical School, San Francisco, Calif, SIDNEY C MADDEN, Emory University School of Medicine, Atlanta, Ga

*Representative in the Division of Medical Sciences of the National Research Council*—(July 1, 1946-June 30, 1949) H P SMITH, College of Physicians and Surgeons, Columbia Univ, New York, N Y

*Representatives on the Council of the American Association for the Advancement of Science*—MALCOLM H SOULE, Univ of Michigan, E B KRUMBHAAR, University of Pennsylvania (terms until June 30, 1949)

*Representative on the Council of the Union of American Biological Societies*—H P SMITH, Columbia Univ, College of Physicians and Surgeons, New York, N Y

*Representatives on the Eli Lilly Award Committee* (Jointly with the Society of American Bacteriologists)—For nominations PAUL R CANNON, University of Chicago For Award PEYTON ROUS, Rockefeller Institute, July 1, 1948-June 30, 1951

*Representative on the Committee for the Placement Service*—DOUGLAS H SPRUNT, Univ of Tennessee Medical School, Memphis, Tenn

*Representative in the Division of Medical Sciences of the National Academy of Sciences*—H P SMITH, Columbia Univ, College of Physicians and Surgeons, New York, N Y

## PAST OFFICERS

1914 R M PEARCE, President, JOHN F ANDERSON, Vice-President, G H WHIPPLE, Secretary-Treasurer, HARVEY CUSHING, DAVID MARINE, Councilors 1915 THEOBALD SMITH, President, G H WHIPPLE, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1916 SIMON FLEXNER, President, LEO LOEB, Vice-President, PEYTON ROUS, Secretary-Treasurer, DAVID MARINE, R M PEARCE, Councilors 1917 LUDVIG HEKTOEN, President, LEO LOEB, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, PAUL A LEWIS, L G ROWNTREE, Councilors 1918 H GIDEON

WELLS, President, W G MACCALLUM, Vice President, HOWARD T KARSNER, Secretary-Treasurer, L G ROWNTREE, LUDVIG HEKTOEN, Councilors 1919 W G MACCALLUM, President, WILLIAM H PARK, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, LUDVIG HEKTOEN, E L OPIE, Councilors 1920 WILLIAM H PARK, President, F G NOVY, Vice-President, HOWARD T KARSNER, Secretary-Treasurer, E L OPIE, WADE H BROWN, Councilors 1921 F G NOVY, President, HOWARD T KARSNER, Vice-President, WADE H BROWN, Secretary-Treasurer, PAUL A LEWIS, A R DOCHEZ, Councilors 1922 HOWARD T KARSNER, President, EUGENE L OPIE, Vice-President, WADE H BROWN, Secretary-Treasurer, A R DOCHEZ, GEORGE H WHIPPLE, Councilors 1923 EUGENE L OPIE, President, ALDRED S WARTHIN, Vice President, WADE H BROWN, Secretary-Treasurer, GEORGE H WHIPPLE, H GIDEON WELLS, Councilors 1924 ALDRED S WARTHIN, President, GEORGE H WHIPPLE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, H GIDEON WELLS, FREDERICK L GATES, Councilors 1925 GEORGE H WHIPPLE, President, WADE H BROWN, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, DAVID MARINE, Councilors 1926 WADE H BROWN, President, DAVID MARINE, Vice-President, EDWARD B KRUMBHAAR, Secretary-Treasurer, FREDERICK L GATES, WILLIAM F PETERSEN, Councilors 1927 DAVID MARINE, President, EDWARD B KRUMBHAAR, Vice-President, CARL V WELLER, Secretary-Treasurer, WILLIAM F PETERSEN, FREDERICK L GATES, Councilors 1928 EDWARD B KRUMBHAAR, President, WILLIAM F PETERSEN, Vice-President, CARL V WELLER, Secretary-Treasurer, FREDERICK L GATES, SAMUEL R HAYTHORN, Councilors 1929 WILLIAM F PETERSEN, President, FREDERICK L GATES, Vice-President, CARL V WELLER, Secretary-Treasurer, SAMUEL R HAYTHORN, PEYTON ROUS, Councilors 1930 FREDERICK L GATES, President, SAMUEL R HAYTHORN, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, PEYTON ROUS, CARL V WELLER, Councilors 1931 SAMUEL R HAYTHORN, President, PEYTON ROUS, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, CARL V WELLER, S BURT WOLBACH, Councilors 1932 PEYTON ROUS, President, CARL V WELLER, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, S BURT WOLBACH, OSKAR KLOTZ, Councilors 1933 CARL V WELLER, President, S BURT WOLBACH, Vice-President, C PHILLIP MILLER, Secretary-Treasurer, OSKAR



KLOTZ, ALPHONSE R DOCHEZ, Councilors 1934 S BURT WOLBACH, President, OSKAR KLOTZ, Vice-President, SHIELDS WARREN, Secretary-Treasurer, C PHILLIP MILLER, ALPHONSE R DOCHEZ, Councilors 1935 OSKAR KLOTZ, President, ALPHONSE R DOCHEZ, Vice-President, SHIELDS WARREN, Secretary-Treasurer, MORTON McCUTCHEON, C PHILLIP MILLER, Councilors 1936 ALPHONSE R DOCHEZ, President, C PHILLIP MILLER, Vice-President, SHIELDS WARREN, Secretary-Treasurer, MORTON McCUTCHEON, ERNEST W GOODPASTURE, Councilors 1937 C PHILLIP MILLER, President, MORTON McCUTCHEON, Vice-President, PAUL R CANNON, Secretary-Treasurer, ERNEST W GOODPASTURE, SHIELDS WARREN, Councilors 1938 MORTON McCUTCHEON, President, ERNEST W GOODPASTURE, Vice-President, PAUL R CANNON, Secretary-Treasurer, SHIELDS WARREN, JESSE L BOLLMAN, Councilors 1939 ERNEST W GOODPASTURE, President, SHIELDS WARREN, Vice-President, PAUL R CANNON, Secretary-Treasurer, JESSE L BOLLMAN, BALDUIN LUCKÉ, Councilors 1940 SHIELDS WARREN, President, JESSE L BOLLMAN, Vice-President, H P SMITH, Secretary-Treasurer, BALDUIN LUCKÉ, PAUL R CANNON, Councilors 1941 JESSE L BOLLMAN, President, BALDUIN LUCKÉ, Vice-President, H P SMITH, Secretary-Treasurer, PAUL R CANNON, DOUGLAS H SPRUNT, Councilors 1942, 1943, 1944, 1945 BALDUIN LUCKÉ, President, PAUL R CANNON, Vice President, H P SMITH, Secretary-Treasurer, DOUGLAS H SPRUNT, FRIEDA S ROBSCHT-ROBBINS, Councilors 1946 PAUL R CANNON, President, DOUGLAS H SPRUNT, Vice-President, FRIEDA S ROBSCHT-ROBBINS, Secretary-Treasurer, H P SMITH, JOHN G KIDD, Councilors 1947 DOUGLAS H SPRUNT, President, H P SMITH, Vice President, FRIEDA S ROBSCHT-ROBBINS, Secretary-Treasurer, JAMES F RIVEHART, JOHN G KIDD, Councilors

## CONSTITUTION

### ARTICLE I—Name

The Society shall be named "THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY"

### ARTICLE II—Object

The object of this Society is to bring the productive investigators in pathology, working essentially by experimental methods, in closer affiliation with the workers in the other fields of experimental medicine

### ARTICLE III—Time and Place of Meeting

The Society shall meet at the same time and place as the Federation of American Societies for Experimental Biology, which comprises at present the American Physiological Society, the American

Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, the American Society for Experimental Pathology, the American Institute of Nutrition and the American Association of Immunologists

## ARTICLE IV—Membership

SECTION 1 Any American investigator who, through the use of experimental methods, has, within three years prior to his candidacy, contributed meritorious work in pathology, is eligible to membership

SEC 2 It shall be the policy of the Society to restrict its membership to as small numbers as is compatible with the maintenance of an active existence

SEC 3 There shall be two classes of members active and honorary members

*Active members* Candidates for active membership shall be nominated at or before an annual meeting by two members of the Society. The nominators shall present to the Secretary in writing evidence of the candidate's qualifications for membership. Nominations approved by the Council shall be presented to the Society for election at the next annual meeting following nomination. For election a favorable ballot by a majority of the members present is necessary

*Honorary members* These may be elected from the active list or from the group of distinguished investigators at home or abroad who have contributed to the knowledge of pathology by experimental study. They shall be elected only by the unanimous vote of the members present at time of nomination

SEC 4 Active members shall pay such annual dues as are determined upon, from year to year, by the Council. Honorary members shall pay no dues, are not eligible to office, and have no vote in the business affairs of the Society, but they shall have all the privileges of the active members in the scientific proceedings

SEC 5 Upon failure of an active member to pay dues for two years, notice shall be given to the member by the Secretary. At the end of the third year, if dues are still unpaid, such failure constitutes forfeiture of membership

SEC 6 A motion for expulsion of a member must be thoroughly investigated by the Council, at this investigation the accused shall be afforded a hearing or may be represented by a member. Expulsion can be accomplished only after a unanimous vote by the Council in favor of expulsion, sustained by a four-fifths vote of the members present at the meeting

## ARTICLE V—Officers

The management of the Society shall be vested in a Council of five members, consisting of a Presi-

dent, a Vice-President, a Secretary-Treasurer, and two other members who shall be nominated by the Council and elected by the Society. Officers are elected by a majority vote and remain in office until July 1 following the Federation Meeting. Vacancies shall be filled by the Council for the unexpired term.

The President and Vice-President shall hold office for one year and are ineligible for re-election during the following year. The Secretary-Treasurer is eligible for re-election. Councilors shall hold office for two years and are elected on alternate years. At the first election one Councilor shall be elected for a short term of one year.

#### ARTICLE VI —*Quorum*

SECTION 1 —Three constitute a quorum of the Council. The Council decides by a majority vote.

SEC. 2 A quorum of the Society for transaction of business shall be one-fourth of the total membership. In all questions brought before the Society a majority vote of those present shall decide, except as elsewhere provided for.

#### ARTICLE VII —*Annual Meeting*

SECTION 1 Papers shall be limited to ten minutes. However, on motion and with unanimous consent, the time may be prolonged by a period not exceeding five minutes. The Council may make provision for longer papers on suitable occasions.

SEC. 2 The subjects of papers must be confined to experimental work in pathology. In doubtful cases a liberal interpretation by the President and Secretary may prevail. The Council may invite, however, presentations dealing with any subject

which it considers of considerable interest to the Society.

#### ARTICLE VIII —*Change of Constitution*

A motion concerning a change of the Constitution must be presented to the Council in writing by three members, and must be communicated to the members by the Secretary at least four weeks before the annual meeting. At this meeting such a change may be established when accepted by a four-fifths vote of the members present.

#### BY-LAWS

1 There must be in each year at least one meeting of the Council, which shall take place not later than the evening before the annual meeting.

2 At the end of the first session of the annual meeting the Secretary shall read the report of the Council. This report shall include (1) names of persons recommended for membership, (2) nominations for offices, (3) matters of general interest. The Secretary shall exhibit in a conspicuous place the names of candidates for membership recommended by the Council, together with the evidence of the qualifications of the candidates.

3 The election of officers and of new members, changes in the Constitution, etc., shall be voted upon at the end of the first session.

4 Changes in the By-Laws may be determined by a majority vote of those present.

5 In the year that a new Secretary-Treasurer is elected the incoming Council Member elected that year, or another member of the Council, shall become Assistant Secretary-Treasurer for the duration of the term of the Secretary-Treasurer.

# AMERICAN INSTITUTE OF NUTRITION

*Founded April 11, 1933, Incorporated November 16, 1934*

*Member of Federation 1940*

## OFFICERS, 1948-1949

*President*—E M NELSON, Food and Drug Administration, Federal Security Agency, Washington, D C

*Vice President*—C G KING, Nutrition Foundation, Inc, Chrysler Building, New York City

*Secretary*—J H ROE, George Washington University School of Medicine, Washington, D C

*Treasurer*—N R ELLIS, Bureau of Animal Husbandry, U S Department of Agriculture, Beltsville, Md

*Councilors*—H J ALMQUIST, F E Booth Company Laboratories, Emeryville, Calif, A D HOLMES, University of Massachusetts, Amherst, Mass, E N TODHUNTER, University of Alabama, University, Ala

*Nominating Committee*—H G DAY, *Chairman*, Indiana University, Bloomington, E W CRAMPTON, Macdonald College, McGill University, Quebec, Canada, H J DEUEL, University of Southern California Medical School, Los Angeles, HELEN A HUNSCHER, Western Reserve University, Cleveland, Ohio, F J STARE, Harvard University, Boston, Mass

## PAST-OFFICERS

1933 L B MENDEL, President, H C SHERMAN, Vice-President, J R MURLIN, Secretary-Treasurer, E F DuBois, M S ROSE, Councilors 1934 J R MURLIN, President, E F DuBois, Vice-President, ICIE G MACY, Secretary, W M BOOTHBY, Treasurer, A H SMITH, AGNES FAY MORGAN, R M BETHKE, Councilors 1935 J R MURLIN, President, E F DuBois, Vice President, ICIE G MACY, Secretary, G R COWGILL, Treasurer, A H SMITH, R M BETHKE, L A MAYNARD, Councilors 1936 E F DuBois, President, MARY SWARTZ ROSE, Vice President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, R M BETHKE, L A MAYNARD, C A ELVEHJEM, Councilors 1937 MARY S ROSE, President, E V MCCOLLUM, Vice President, G R COWGILL, Treasurer, ICIE G MACY, Secretary, L A MAYNARD, C A ELVEHJEM, P E HOWE, Councilors 1938 E V MCCOLLUM, President, T M CARPENTER, Vice President, G R COWGILL, Treasurer, L A MAYNARD, Secretary, C A ELVEHJEM, P E HOWE, HELEN S MITCHELL, Councilors 1939 H C SHERMAN, President, T M CARPENTER, Vice President, G R COWGILL, Treasurer, L A MAYNARD, Secretary, P E HOWE, HELEN S MITCHELL, A H SMITH, Councilors 1940

THORNE M CARPENTER, President, A G HOGAN, Vice President, L A MAYNARD, Secretary, W H SEBRELL, JR, Treasurer, HELEN S MITCHELL, ARTHUR H SMITH, LYDIA J ROBERTS, Councilors 1941 A G HOGAN, President, L A MAYNARD, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR, Treasurer, T H JUKES, LYDIA J ROBERTS, H B LEWIS, Councilors 1942 L A MAYNARD, President, H B LEWIS, Vice-President, ARTHUR H SMITH, Secretary, W H SEBRELL, JR, Treasurer, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1943 H B LEWIS, President, ICIE G MACY-HOOBLER, Vice-President, ARTHUR H SMITH, Secretary, LYDIA J ROBERTS, GENEVIEVE STEARNS, T H JUKES, Councilors 1944 ICIE G MACY-HOOBLER, President, Wm C ROSE, Vice-President, ARTHUR H SMITH, Secretary, E M NELSON, Treasurer, GENEVIEVE STEARNS, T H JUKES and C A ELVEHJEM, Councilors 1945 Wm C ROSE, President, ARTHUR H SMITH, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, T H JUKES, C A ELVEHJEM, D W WOOLLEY, Councilors 1946 ARTHUR H SMITH, President, R M BETHKE, Vice-President, H E CARTER, Secretary, E M NELSON, Treasurer, C A ELVEHJEM, D W WOOLLEY, H J ALMQUIST, Councilors 1947 R M BETHKE, President, E M NELSON, Vice-President, H E CARTER, Secretary, N R ELLIS, Treasurer, D W WOOLLEY, H J ALMQUIST, A D HOLMES, Councilors

## CONSTITUTION

1 The name of the proposed society is the "AMERICAN INSTITUTE OF NUTRITION"

2 The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest

3 The management of the American Institute of Nutrition shall be vested in a council consisting of the President, Vice-President, Secretary Treasurer and three additional members

## BY-LAWS

### ARTICLE I—Membership

SECTION 1 *Eligibility for membership* Members Qualified investigators who have independently conducted and published meritorious original investigations in some phase of the chemistry or physiology of nutrition and who have shown a professional interest in nutrition for at least 5

years shall be eligible for membership in the Society

**SEC 2 *Nomination*** Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting

**SEC 3 *Election to membership*** A A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility B A majority of the ballots cast shall elect

**SEC 4 *Forfeiture*** If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society

#### ARTICLE II—*Meetings and Quorum*

**SECTION 1 *Annual*** The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation

**SEC 2 *Special*** A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifty members of the Society. Notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held

**SEC 3 *Quorum*** Thirty members shall constitute a quorum at all meetings of the Society, but in the absence of a quorum any number shall be sufficient to adjourn to a fixed date

#### ARTICLE III—*Officials*

**SECTION 1 *Officers*** The officers shall be a President, and a Vice-President, who shall be elected annually, and a Secretary and Treasurer, each of whom shall be elected to serve for a term of three years. These officers shall be elected by the members of the Society. Their terms of office shall commence on July 1 of the year in which they are elected

**SEC 2 *Council*** The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and

shall be known as 'The Council' (When this provision is first put into effect one member shall be elected for 1 year, one for 2 years and the third for 3 years)

**SEC 3 *Duties of Officers*** The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions

#### ARTICLE IV—*The Council*

**SECTION 1 *Powers*** The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York. A permanent charter was issued to the American Institute of Nutrition under date of November 16, 1934

**SEC 2 *Reports*** The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws

#### ARTICLE V—*Nominating Committee*

**SECTION 1 *Membership*** A The Nominating Committee shall consist of five members appointed for the coming year by the retiring President. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for reappointment until after a lapse of one year B The President shall designate one member to be Chairman of the Nominating Committee

**SEC 2 *Nomination of Officials*** A The Nominating Committee shall make at least one nomination for each of the four offices, for each of the additional positions on the Council to be filled by vote of the members and for each of the positions on the Editorial Board to be vacated at the time of the annual meeting. Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee B The nominations by the Nominating Committee shall be transmitted to the Secretary at least six weeks before the annual meeting at which they are to be considered C The Secretary shall send to every member, at least two weeks before the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail, returning to the Secretary the marked ballot in the envelope provided, at such a time and place as the Secretary may designate, or the ballot may be delivered to the Secretary at the beginning of the business session at which the elections are to take place

SEC 3 *Election of Officials* A At the beginning of the business session the Secretary shall present to the tellers, appointed by the President, the ballots submitted by the members and the ballots shall be counted forthwith B A majority of votes cast shall be necessary to elect an official

SEC 4 *Filling of Vacancies* A The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society B The President of the Society shall fill all vacancies in appointive positions

#### ARTICLE VI—*Financial*

SECTION 1 *Dues* The dues shall be the annual cost of subscription to The Journal of Nutrition for members plus an annual assessment which shall be determined by majority vote at the annual meetings, upon recommendation of the Council, and shall be due within a month after the annual meeting A member on attaining the age of 65 may elect to be relieved from all financial obligations to the Institute including subscription to The Journal of Nutrition

SEC 2 *Expenditures* No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council

SEC 3 *Penalty for non-payment of dues* A Members in arrears for dues for two consecutive years shall forfeit their membership B Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated

#### ARTICLE VII—*The Journal of Nutrition*

SECTION 1 The American Institute of Nutrition designates The Journal of Nutrition as its official organ of publication

SEC 2 In accordance with the expressed wish of the Wistar Institute of Anatomy and Biology, owner and publisher of The Journal of Nutrition, the American Institute of Nutrition shall nominate members of the Editorial Board for its official

organ A The editorial management of The Journal of Nutrition shall be vested in an Editorial Board consisting of an Editor and twelve Board Members B The Editor shall be chosen by the Editorial Board to serve a term of five years beginning July 1 of the year in which he is chosen, and shall be eligible for reelection The Editor shall have the power to designate one of the Board Members to serve as his assistant, and such an appointee shall be called Associate Editor C Three members of the Institute shall be nominated by the Nominating Committee for membership on the Editorial Board each year to serve a term of four years, replacing three retiring members and taking office May 1 of the year in which they are elected In the event of a vacancy in the membership of the Editorial Board occurring through death or other reason, the Nominating Committee, for each such vacancy to be filled shall make an additional nomination In this event the nominees elected who receive the greatest number of votes shall serve the longest term of vacancies to be filled D Retiring members of the Editorial Board shall not be eligible for renomination until one year after their retirement

#### ARTICLE VIII—*Papers on Scientific Subjects*

SECTION 1 The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings

#### ARTICLE IX—*Changes in Constitution and By-Laws*

SECTION 1 Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three members The Secretary shall send a printed copy of any proposed change to each member at least two weeks before the next meeting and shall notify all members that they may vote by proxy

SEC 2 If at this meeting two thirds of the votes cast shall favor the proposed change, it shall be made

# THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

*Founded June 19, 1913, Member of Federation 1942*

## OFFICERS, 1948-1949

*President*—MICHAEL HEIDELBERGER, Columbia University College of Physicians and Surgeons

*Vice-President*—LLOYD D. FULTON, National Institutes of Health, Bethesda, Md

*Secretary-Treasurer*—JULIUS FREUND, Public Health Research Institute of the City of New York, New York City

*Honorary President*—ARTHUR F. COCA, Lederle Laboratories, Pearl River, N. Y.

*Council*—JACQUES J. BRONFENBRENNER, PAUL R. CANNON, GEOFFREY EDSALL, KARL F. MEYER, SANFORD B. HOOKER, *ex officio*, THOMAS FRANCIS, JR.

## PAST OFFICERS

*Presidents*—1913 GERALD B. WEBB 1915 JAMES W. JOBLING 1916 RICHARD WEIL 1917 JOHN A. KOLMER 1918 WILLIAM H. PARK 1919 HANS ZINSSER 1920 RUFUS I. COLE 1921 FREDERICK P. GAY 1922 GEORGE W. MCCOY 1923 H. GIDEON WELLS 1924 FREDERICK G. NOVY 1925 WILFRED H. MANWARING 1926 LUDVIG HEKTOEN 1927 KARL LANDSTLINER 1928 EUGENE L. OPIE 1929 OSWALD T. AVERY 1930 STANHOPE BAYNE-JONES 1931 ALPHONSE R. DOCHETZ 1932 AUGUSTUS B. WADSWORTH 1933 THOMAS M. RIVERS 1934 FRANCIS G. BLAKE 1935 WARFIELD T. LONGCOPE 1936 SANFORD B. HOOKER 1937 CARL TENBROECK 1938 DONALD T. FRASER 1939 GEORGE P. BERRY 1940 PAUL R. CANNON 1941 KARL F. MEYER 1942-1945 JACQUES J. BRONFENBRENNER 1945-1947 MICHAEL HEIDELBERGER 1947 LLOYD D. FULTON

*Vice-Presidents*—1913-1915 GEORGE W. ROSS 1915 GEORGE P. SANBORN 1916 JOHN A. KOLMER 1947 MICHAEL HEIDELBERGER

*Secretary*—1913-1918 MARTIN J. SYNOTT

*Treasurer*—1913-1918 WILLARD J. STONE

*Secretary-Treasurer*—1918-1947 ARTHUR F. COCA

## CONSTITUTION

*(As revised May, 1947)*

### ARTICLE I

SECTION 1 This Association shall be called THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

SECTION 2 The object of the Association shall be to promote the knowledge of immunology, chemotherapy, virology and related disciplines, and to facilitate contact between investigators in those and related fields

### ARTICLE II

SECTION 1 The Association shall be governed by a Council which shall consist of the Officers of the Association, four Councillors, and a representative of the Board of Editors of the Journal of Immunology

SECTION 2 The Officers of the Association shall be a President, a Vice President, a Secretary, and a Treasurer

SECTION 3 The President, the Secretary, and the Treasurer shall be elected at the regular annual meeting of the Association to serve for one year. They shall take office the day after the end of the annual meeting

SECTION 4 The President shall not serve for more than one year consecutively. The Secretary and the Treasurer are eligible for reelection

SECTION 5 The outgoing President shall serve as Vice President for the year subsequent to his Presidency

SECTION 6 The Editors of the Journal of Immunology shall designate annually one out of their number as their representative with power to vote in the Council of the Association

SECTION 7 One Councillor shall be elected each year to serve for four years. No Councillor may be reelected until one year after expiration of his term. He may, however, serve in any other elective office immediately after expiration of his term as Councillor

SECTION 8 The President shall appoint a Nominating Committee of three (or more) members not holding executive office in the Association and shall designate the Chairman. The Nominating Committee shall make at least one nomination for each of the offices. Nominations made by the Nominating Committee shall be transmitted to the Secretary at least six weeks before the annual meeting. The Secretary shall send to every member of the Association, at least four (4) weeks before the annual meeting, a ballot containing the list of the nominees and spaces for such additional nominees as the members might wish to propose

SECTION 9 The members may vote by mail. Ballots sent by mail must be in the hands of the Secretary before the opening of the annual meeting. Alternatively, members may vote at the annual meeting. At the annual meeting the Secretary shall present to tellers appointed by the President, all ballots received by him

SECTION 10 A plurality of votes shall be sufficient for election

SECTION 11 It is the duty of the Council to conduct the business of the Association

SECTION 12 Should a vacancy occur in the Council other than by expiration of term of service, the Council may elect a member to fill the vacancy until the next regular meeting

SECTION 13 The Vice President shall substitute for the President when necessary. If a vacancy should occur in the offices of the Secretary or Treasurer the Council may elect a member to fill the vacancy for the unexpired portion of the term

SECTION 14 In case of equal division of votes, the President shall cast the decisive ballot

### ARTICLE III

SECTION 1 The Association shall consist of active members, members emeriti, and honorary members

SECTION 2 Any qualified person engaged in the study of problems related to the purpose of the Association shall be eligible to active membership

SECTION 3 Candidates for active membership shall be nominated by two members of the Association on blanks furnished by the Secretary. Applications must be accompanied by letters of recommendation of the sponsors, a curriculum vitae, reprints of publication and other suitable evidence of fitness. Nominations are to be submitted to the Council which shall determine eligibility and shall post a list of candidates at the annual meeting. The membership shall elect new members by majority vote

SECTION 4 Failure to pay dues for three successive years shall annul membership. However, the Council may reinstate a member if an acceptable explanation is submitted

SECTION 5 If a 2/3 majority of the Council decides that the interests of the Society require the expulsion of a member, the Secretary shall notify the affected member in writing of the charges. The Council shall allow a reasonable time for the presentation of his defence before acting. Upon recommendation of a 2/3 majority of the Council, the Secretary shall send a notice of the decision to each active member at least three weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion, and on a two thirds vote of the members present, the member shall be expelled, his assessment for the current year shall be returned and he shall cease to be a member of the Society

SECTION 6 A member on attaining the age of sixty five years may elect to accept the status of member emeritus. As such he shall retain voting privileges but shall be relieved of all financial obligations to the Association including subscription to the Journal of Immunology and the Federation Proceedings

SECTION 7 The Council may nominate for honorary membership persons of extraordinary achievement in the field of endeavor of this Association. Election to honorary membership shall follow the same procedure as that for election for office in the Association, and the Secretary, on order of the Council shall place nominations for honorary membership on the annual ballot

### ARTICLE IV

SECTION 1 The members present at the annual meeting of the Association shall constitute a quorum

SECTION 2 A quorum of the Council for the transaction of all business shall be three

### BY-LAWS

1 A regular meeting of the Association shall be held annually at such time and place as the Council shall determine

2 Special meetings of the Association may be held at the discretion of the Council

3 Regular and special meetings shall be open to all members of the Association

4 A meeting of the Council shall be held before each annual session of the Association

5 The Past Presidents shall have the right of attending, without vote, the meetings of the Council

6 The President may appoint a Past President as pro tempore Councilor at any stated meeting of the Council at which a quorum is not present

7 The Council may transact, and vote by mail on, such business as cannot be conveniently transacted at meetings

8 The fiscal year of the Association shall begin April first

9 The Secretary shall arrange the program for the scientific meetings, with the advice of the other officers of the Association. Papers intended for presentation at the meetings shall conform to the standards of the Journal of Immunology. In case of doubt, the Secretary shall have the right to submit papers to the scrutiny of two members of the Editorial Board of the Journal of Immunology, whose decision shall be final

10 The dues of the Association shall be determined annually by the Council and shall include subscription to the Journal of Immunology

11 Proposed changes in the Constitution and By-Laws must be submitted in writing to the Secretary. The President shall then appoint a Committee of at least three members which shall communicate its recommendations to the Secretary

12 Recommendations of such Committees shall be sent to the membership of the Association by the Secretary with the annual ballot. A change in the Constitution and the By Laws shall require

a two-thirds majority of the members casting votes either by mail or at the annual meeting

13 The Journal of Immunology, which is the property and official organ of this Association, shall be administered for the Association by an Editorial Staff

14 The Editorial Staff shall be organized and its members shall be elected by or may be removed by a majority vote of the Council of the Association

15 If by force of circumstances it should be impossible to have an annual meeting, election of Officers and Council may be held entirely by mail. If this also should prove to be impossible, the Council may direct the Officers to continue in their Offices until such time as elections can be held

16 The Council shall adopt temporary rules for the transition period after the adoption of the amended Constitution



## ALPHABETICAL LIST OF ALL MEMBERS OF THE SIX SOCIETIES

The parenthesis following each listed name gives the Society affiliation and year of election

- (1) The American Physiological Society
- (2) American Society of Biological Chemists
- (3) The American Society for Pharmacology and Experimental Therapeutics
- (4) The American Society for Experimental Pathology
- (5) The American Institute of Nutrition
- (6) The American Association of Immunologists

### HONORARY MEMBERS

- Adrian, E D Dept of Physiology, Cambridge University, Cambridge, England (1, 1946)
- Castaneda, M Ruiz, M D Investigaciones Medicas, Hospital General, Mexico, D F *Director, Department of Medical Research* (6, 1942)
- Chopra, R N, M A, M D, Sc D (Cantab), F R C P (London) P I E School of Tropical Medicine, Calcutta, India *Director, Professor of Pharmacology* (3, 1938)
- Coca, Arthur F, A M, M D Oradell, N J (6, 1916)
- Dale, H H The Wellcome Trustees, Dilke House, Malet St, London, W C, England (3, 1926)
- Hektoen, Ludvig, M D 629 S Wood St, Chicago, Ill *President, Chicago Tumor Institute* (6, 1919)
- Hitchens, Arthur P, M D Public Building, Wilmington 33, Del *Health Commissioner, Wilmington* (6, 1913)
- Houssay, Bernardo A, M D Viamonte 2790, Buenos Aires, Argentina *Director, and Professor of Physiology* (1, 1942)
- Huntoon, F M, M D Woodbridge, Conn (6, 1918)
- Krogh, August Juliane Marariesvej 34, Copenhagen, Denmark (1, 1946)
- Lapicque, L Laboratory of Physiology, The Sorbonne, Paris, France (1, 1946)
- Loewi, Otto, M D New York University College of Medicine, 477 First Ave, New York City *Research Professor in Pharmacology* (3, 1941)
- McCoy, George Walter, M D Louisiana State University Medical School, New Orleans *Director, Department of Public Health* (6, 1916)
- Novy, Frederick G, M D, Sc D, LL D 721 Forest Ave, Ann Arbor, Mich *Dean Emeritus and Professor Emeritus of Bacteriology, Medical School, University of Michigan* (6, 1920)
- Orbeli, L A Academy of Sciences of the USSR, Moscow, USSR (1, 1946)
- Porter, William Townsend, M D, Sc D, LL D Dover, Mass *Emeritus Professor of Comparative Physiology, Harvard University* (1, 1891)
- Sherrington, Sir Charles S, O M, Sc D, M D,

F R S "Broomside," Valley Road, Ipswich, England *Former Wayneffe Professor of Physiology, Oxford University, Former President of the Royal Society* (1, 1904)

Sordelli, A Institute of Bacteriology, Department of Public Health, Buenos Aires, Argentina *Director* (6, 1942)

### RETIRED MEMBERS

Addison, William H, M D University of Pennsylvania, Philadelphia, Pa *Professor of Histology and Embryology* (1, 1928)

Allen, William F, Ph D, D Sc University of Oregon Med Sch, Portland *Emeritus Prof of Anatomy* (1, 1929)

Babkin, B P, M D, D Sc, F R S C McGill University, Montreal, Canada *Prof of Physiology* (1, 1924)

Bachmann, George, M D, F A C P Emory University School of Med, Emory University, Ga *Emeritus Prof of Physiology* (1, 1912)

Brown, Edgar D, Pharm D, M D Paynesville, Minn *Emeritus Assoc Prof of Pharmacology* (1, 1907, 3, 1909)

Burton-Opitz, Russell, Ph D 218 Bridle Way, Palisade, N J *Attending Cardiologist, Lenox Hill Hospital, Attending Physician, Cumberland Hospital, Consulting Cardiologist Englewood, North Hudson, Holy Name and Hackensack Hospitals* (1, 1902, 3, 1919)

Campbell, H Louise, Ph D 900 Windsor Ave, Windsor, Conn (5, 1933)

Child, Charles M, Ph D, D Sc (hon) Stanford University, Calif *Member, Nat'l Academy of Sciences, Emeritus Prof, University of Chicago* (1, 1923)

Clark, Eliot R, M D University of Pennsylvania, Philadelphia, Pa *Prof and Head of Dept of Anatomy* (1, 1919)

Coca, Arthur F, M D Lederle Laboratories, Pearl River, N Y *Medical Director* (6, 1916)

Culler, Elmer A, Ph D University of Rochester, Rochester, N Y *Prof of Psychology and Director of the Lab* (1, 1936)

Dawson, Percy M, M D 665 E Maryland Ave, Claremont, Calif (1, 1900)

- Dooley, M S**, M D Syracuse Univ College of Med, Syracuse, N Y *Prof of Pharmacology* (3, 1923)
- Durrant, Edwin P**, Ph D Ohio State University, Columbus, Ohio *Emeritus Assoc Prof of Physiology* (1, 1928)
- Erlanger, Joseph**, M D, LL D, Sc D Washington Univ Sch of Med, St Louis, Mo *Emeritus Prof of Physiology Member of the Natl Academy of Sciences* (1, 1901)
- Famulener, Lemuel W**, Ph C, M D 275 Ingle St, Englewood, N J (6, 1920)
- Fitzgerald, Mabel P**, 54 A George Sq Edinburgh, Scotland (1, 1913)
- Forbes, Henry S**, M D Harvard Medical School, Boston, Mass *Assoc in Neuropathology* (1, 1931)
- Githens, Thomas S**, M D The Cambridge Alden Park, Wissahickon and School Lane, Germantown, Philadelphia, Pa (1, 1915)
- Glaser, O C**, Ph D Amherst College, Amherst, Mass *Prof of Biology* (1, 1913)
- Hale, Worth**, M D Antrim, New Hampshire (1, 1908, 3, 1908)
- Halsey, John T**, M D P O Box 264, Waveland, Miss *Emeritus Prof of Pharmacology, Tulane Univ* (3, 1929)
- Herrick, C Judson** Ph D University of Chicago, Chicago, Ill *Emeritus Prof of Neurology, Member of the Natl Academy of Sciences* (1, 1907)
- Hoskins, R G**, Ph D M D 86 Varick Rd, Waban 68, Mass (1, 1911)
- Knowlton, Frank P**, M D Syracuse University Col of Med, Syracuse, N Y *Emeritus Prof of Physiology* (1, 1911)
- Kyes, Preston**, Sc D, M D University of Chicago, Chicago, Ill *Emeritus Prof* (6, 1918)
- Laurens, Henry D** Ph D, M D The Rockefeller Institute, New York City *Associate* (1, 1913)
- Lewis, Warren H**, M D Wistar Institute of Anatomy and Biology, Philadelphia, Pa *Member, Member of the Natl Academy of Sciences* (1, 1919)
- Loebel, Robert O**, M D 205 East 78th St, New York City (1, 1928)
- Mackenzie, George M**, M D Mary Imogene Bassett Hospital, Cooperstown, N Y *Physician-in-Chief, Director, Otsego County Lab* (6, 1912)
- Moulton, C Robert**, Ph D 5602 Dorchester Ave, Chicago, Ill (5, 1933)
- Parker, George H**, Sc D 16 Berkeley St, Cambridge, Mass *Emeritus Professor of Zoology, Harvard Univ, Member of the Natl Academy of Sciences* (1, 1909)
- Pilcher, J Douglas**, M D City Hospital, Scranton Rd, Cleveland, Ohio *Assoc Prof of Pediatrics, Western Reserve Univ School of Medicine* (3, 1911)
- Pohlman, Augustus G**, M D University of Southern California, Los Angeles, Calif *Assoc Prof Dept of Otolaryngology* (1, 1934)
- Pratt, Frederick H**, M D Boston University Sch of Med, Boston, Mass *Emeritus Prof of Physiology* (1, 1919)
- Quinby, William C**, M D Harvard University Med Sch, Boston, Mass *Clin Prof of Genito urinary Surgery* (1, 1916)
- Riddle, Oscar**, Ph D Cold Spring Harbor, L I, N Y *Visiting Prof from the U S (in South America) Member of the Natl Academy of Sciences* (1, 1919)
- Rogers, Charles G**, Ph D, Sc D Oberlin College, Oberlin, Ohio *Prof of Comparative Physiology* (1, 1911)
- Roth, George B**, M D 3811 T St, N W, Washington, D C *Emeritus Prof of Pharmacology, Geo Washington Univ* (1, 1914, 3, 1911)
- Sabin, Florence R**, M D, Sc D 1333 E 10th Ave, Denver 3, Colo *Emeritus Member, Rockefeller Inst, Member of National Academy of Sciences* (1, 1923)
- Sacks, Ernest**, M D 97 Arundel Pl, St Louis, Mo *Emeritus Prof of Clin Neurological Surgery, Washington Univ Med Sch* (1, 1910)
- Sappington, Samuel W**, M D, D Sc P O Box 51, Bryn Mawr, Pa *Prof of Pathology, Hahnemann Hospital* (6, 1913)
- Schultz, W H**, Ph D 3102 18th St, N W, Washington, D C *Emeritus Prof of Pharmacology, Univ of Maryland* (1, 1907, 3, 1909)
- Smith, Sybil L**, A M 1421 44th St, N W, Washington, D C
- Snyder, Charles D** Ph D 4709 Keswick Rd, Baltimore, Md *Emeritus Prof of Exptl Physiology, Johns Hopkins Univ* (1, 1907)
- Sweet, J E**, M D, Sc D Unadilla, N Y *Emeritus Prof of Surgical Res, Cornell Univ Med Col* (1, 1913)
- Walker, Ernest Linwood**, S D 50 Winchester St, San Francisco, Calif (3, 1931)
- Wood, Horatio C, Jr**, M D Ph M 319 S 41st St, Philadelphia 4, Penna *Professor of Pharmacology and Therapeutics, Univ of Pennsylvania, Professor of Materia Medica, Philadelphia College of Pharmacy and Science* (3, 1908)
- Wulzen, Rosalind**, Ph D Oregon State College, Corvallis *Asst Prof of Zoology* (1, 1916)
- Yerkes, Robert M**, Ph D Yale Laboratories of Primate Biology, New Haven, Conn *Prof of Psychobiology, Yale Univ, Member of the Natl Academy of Sciences* (1, 1904)

#### MEMBERS

- Abbott, Lynn D F**, Ph D Medical College of Virginia, Richmond, Va *Assoc Professor of Biochemistry* (2, 1948)
- Abramson, David I**, M D Department of Medi-

- cine, University of Illinois, Chicago 12 Assistant Clinical Professor, Attending Physician, Hines Veterans Hospital, Associate Physician, Michael Reese Hospital (1, 1937)
- Abramson, Harold A**, M D 133 E 58th St, New York City Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University (1, 1930, 2, 1934)
- Abreu, Benedict E**, M S, Ph D, Division of Pharmacology, Univ of California Medical School, San Francisco Assistant Professor of Pharmacology (3, 1941)
- Acheson, George H**, M D Univ of Cincinnati, College of Medicine, Cincinnati, Ohio Head, Dept of Physiology (1, 1942, 3, 1945)
- Adams, Georgian, M A**, D Sc United States Department of Agriculture, Washington 25, D C Senior Experiment Station Administrator, Office of Experiment Stations (5, 1946)
- Adams, John M**, M D Department of Pediatrics, W 205 University Hospital, University of Minnesota, Minneapolis Associate Professor of Pediatrics (4, 1947)
- Adams, Mildred, M A**, Ph D Takamine Laboratory, Clifton, N J Research Chemist (2, 1934)
- Adams, R Charles**, M D, C M, M S (Anesthesiology), Mayo Clinic, Rochester, Minn Instructor in Anesthesia, Mayo Foundation, University of Minnesota Member of Mayo Clinic Staff, Section on Anesthesia (3, 1942)
- Adams, W Lloyd**, M D, Ph D U S Public Health Service Hospital, Lexington, Ky Chief of EENT Service and Personnel Physician (3, 1942)
- Adams, Wright R**, B S, M D Dept of Medicine, University of Chicago, Chicago 37, Ill Associate Professor of Medicine (1, 1946)
- Addis, Thomas, M D**, M R C P Lane Hospital, San Francisco, Calif Professor of Medicine, Stanford University (1, 1922)
- Ades, Harlow Whiting**, Ph D Box 734, Emory University, Ga (1, 1945)
- Adler, Harry F**, M S, Ph D, M D Chief, Dept of Physiology, School of Aviation Medicine, Randolph Field, Texas (1, 1943)
- Adolph, Edward Frederick**, Ph D School of Medicine and Dentistry, University of Rochester, Rochester, N Y Professor of Physiology (1, 1921)
- Adolph, William H**, Ph D Peiping Union Medical College, Peiping, China Professor of Biochemistry (2, 1946, 5, 1934)
- Ahlquist, Raymond P**, M S, Ph D Dept of Pharmacology, Univ of Georgia School of Medicine, Augusta Professor and Chairman of the Dept of Pharmacology (3, 1945)
- Albanese, Anthony A**, Ph D G 7 Tower Laby, Children's Medical Service, Bellevue Hospital, New York, N Y Associate Professor of Pediatric Biochemistry, New York University College of Medicine (2, 1944)
- Albaum, Harry G**, M Sc, Ph D Brooklyn College, Bedford Avenue and Avenue H, Brooklyn, N Y Assistant Professor of Biology (2, 1947)
- Albert, A**, M A, Ph D, M D Mayo Foundation, Rochester, Minn Research Associate (1, 1947)
- Albritton, Errett C**, M D George Washington University Medical School, 1339 H St, N W, Washington, D C Professor of Physiology and Head of the Department of Physiology (1, 1933)
- Alexander, Robert S**, A B, M A, Ph D School of Medicine, Western Reserve Univ, 2109 Adelbert Road, Cleveland, Ohio Instructor in Physiology (1, 1946)
- Algire, Glenn H**, M D National Cancer Institute, Bethesda, Md Senior Assistant Surgeon, U S Public Health Service (4, 1945)
- Allan, Frank N**, M D Lahey Clinic, 605 Commonwealth Ave, Boston, Mass Executive Director of the Medical Department (4, 1930)
- Allen, Charles Robert**, Ph D University of Texas, School of Medicine, Galveston Assistant Professor of Department of Anesthesiology (1, 1943)
- Allen, Frank W**, Ph D 1557 Life Science Building, University of California, Berkeley, Calif Associate Professor (2, 1947)
- Allen, Frederick M**, M D 1031 Fifth Ave, New York City Professor of Medicine, Polytechnic Medical School and Hospital (1R, 1924, 4, prior to 1920)
- Allen, J Garrott**, M D University of Chicago, University Clinics, Chicago, Ill Instructor in Surgery (1, 1943)
- Allen, Lane**, M S, Ph D, M D University of Georgia School of Medicine, University Place, Augusta Associate Professor of Anatomy (1, 1939)
- Allen, Shannon C**, Ph D Cornell University, Public Health and Preventive Medicine, New York City (1, 1945)
- Allen, Thomas H**, Ph D College of Physicians and Surgeons, Columbia University, 630 168th St, New York 32, N Y Instructor in Physiology (1, 1947)
- Allen Willard M**, M D Washington University School of Medicine, 630 S Kingshighway Blvd, St Louis, Mo Professor of Obstetrics and Gynecology (1, 1934)
- Alles, Gordon A**, M S, Ph D 770 S Arroyo Parkway, Pasadena, Calif Lecturer in Pharmacology, University of California Medical School, San Francisco, and Research Associate in Biology, California Institute of Technology, Pasadena (1, 1932, 3, 1941)
- Alling, Eric L**, M D School of Medicine and

- Dentistry, University of Rochester, Rochester 7, N Y *Associate in Radiology* (4, 1947)
- Allison, James B, Ph D Rutgers Univ, New Brunswick, New Jersey *Director, Bureau of Biological Research* (2, 1946)
- Almquist, Herman J, Ph D F E Booth Co Laboratories, 1290 Powell St, Emeryville, Calif *Director of Research* (2, 1937, 5, 1937)
- Altura-Werber, Erna Jewish Hospital of Brooklyn, Brooklyn, N Y *Director, Research Laboratory* (6, 1948)
- Alvarez, Walter C, M D Mayo Clinic, Rochester, Minn *Professor of Medicine, Mayo Foundation* (1, 1917, 3R, 1921)
- Alving, Alf Sven, M D Billings Hospital, University of Chicago, 950 E 59th St, Chicago, Ill *Associate Professor of Medicine* (1, 1939)
- Amberg, Samuel, M D, F A A P Mayo Clinic, Rochester, Minn *Associate in Pediatrics, Associate Professor of Pediatrics, Mayo Foundation* (1R, 1903, 2, 1906, 3R, 1909)
- Amberson, William R, Ph D University of Maryland School of Medicine, Baltimore *Professor of Physiology* (1, 1924)
- Ambrose, Anthony M, M S, Ph D Western Regional Research Laboratory, 800 Buchanan St, Albany, Calif *Pharmacologist, U S Department of Agriculture, Bureau of Agricultural Chemistry and Engineering* (3, 1937)
- Ames, Stanley R, Ph D Distillation Products, Inc, Rochester, N Y *Senior Research Chemist* (2, 1948)
- Amoss, Harold L, M D M S, Dr P H, Sc D 68 Deerfield Drive, Greenwich, Conn (4, 1922, 6, 1917)
- Andersch, Marie A, Ph D University Hospital, Baltimore, Md *Biochemist, University Hospital, Instructor in Medicine, University of Maryland* (2, 1940)
- Andersen, Dorothy H, M D Babies Hospital, Broadway and 167th St, New York City *Assistant Professor of Pathology, Columbia University* (4, 1935)
- Anderson, Evelyn M, M A, M D 7206 Blair Rd, N W, Washington 12, D C (1, 1934)
- Anderson, Hamilton H, M S, M D Pharmacology Laboratory, Univ of California Medical School, San Francisco *Professor of Pharmacology* (3, 1931)
- Anderson, Oscar Daniel, Ph D Dept of Psychology, Cornell University, Ithaca, N Y (1, 1939)
- Anderson, Rubert S, Ph D Univ of South Dakota Sch of Med, Vermillion, S D *Professor of Physiology* (1, 1948)
- Anderson, Rudolph J, Ph D Sterling Laboratory, Yale University, New Haven, Conn *Professor of Chemistry* (2, 1915)
- Anderson, W A D, M A, M D Marquette University School of Medicine, Milwaukee, Wis *Professor of Pathology and Bacteriology* (4, 1941)
- Anderson, William E, M A Eastern State Farmers' Exchange, Westbrook Farm, Rockville, Conn *Biochemist* (2, 1931, 5, 1933)
- Andervont, H B, Sc D National Cancer Institute, Bethesda, Md *Biologist, U S Public Health Service* (4, 1939)
- Andrews, James C, Ph D University of North Carolina, Chapel Hill *Professor of Biological Chemistry and Nutrition* (2, 1925)
- Andrus, E Cowles, M D 24 E Eager St, Baltimore 2, Md *Assistant Visiting Physician, Associate Professor of Medicine, Johns Hopkins University* (1, 1925)
- Anfinsen, Christian B, Jr, M S, Ph D Harvard Univ Med Sch, Boston, Mass *Associate in Biological Chemistry* (2, 1946)
- Angerer, Clifford, Ph D Ohio State University, Columbus *Associate Professor of Physiology* (1, 1943)
- Angevine, D Murray, M D Univ of Wisconsin Medical School, Madison, Wis *Professor of Pathology* (1, 1940)
- Ansbacher, Stefan, M S, D Sc 17 Loel Court, Rockville Centre, N Y (2, 1939)
- Anslow, W Parker, Jr, Ph D New York University College of Medicine, New York City *Asst Professor of Physiology* (2, 1948)
- Anson, Mortimer L, Ph D Continental Foods, Inc, Hoboken, N J *Director of Chemical Research* (2, 1937)
- Apperly, Frank L, M A, D Sc, M D, F R C P Medical College of Virginia, Richmond *Professor of Pathology* (4, 1936)
- Archibald, Reginald M, M A, Ph D, M D The Rockefeller Institute for Medical Research, New York, New York (2, 1947)
- Arkin, Aaron, M A, M D, Ph D Suite 2006, 25 E Washington St, Chicago, Ill *Rush Professor of Medicine, U of Ill Prof and Chairman, Dept of Medicine, Cook County Graduate School* (1, 1914, 3, 1919)
- Armstrong, Harry G, M D Randolph Air Force Base, Randolph Field, Texas *Brig Gen 4F, Sch of Aviation Med, Comdt* (1, 1948)
- Armstrong, Philip B, M D College of Medicine, Syracuse Univ, Syracuse 10, N Y *Professor of Anatomy* (1, 1945)
- Armstrong, W D, M S, M D, Ph D 17 Medical Sciences Bldg, University of Minnesota, Minneapolis *Professor and Head of Physiological Chemistry* (2, 1938)
- Arnold, Aaron, M S, Ph D Sterling-Winthrop Research Institute, Rensselaer, N Y *Head of Nutritional Research Laboratory* (5, 1947)
- Arnold, Lloyd, A M, M D 1538 E 57th St, Chicago, Ill (4, 1930, 6, 1925)
- Arnow, L Earle, Ph D, M D Medical Research

- Division, Sharp and Dohme, Glenolden, Pa  
*Director of Research* (2, 1940)
- Aronson, Joseph D, M D Phipps Institute, University of Pennsylvania, Philadelphia 4  
*Associate Professor of Bacteriology* (4, 1927, 6, 1925)
- Arton, Camillo, M D Bowman Gray School of Medicine, Winston-Salem, N C *Professor of Biochemistry* (2, 1944)
- Ascham, Leah, Ph D Kansas State College, Manhattan *Professor, School of Home Economics* (5, 1935)
- Asenjo, Conrado F, Ch E, M S, Ph D School of Tropical Medicine, San Juan, Puerto Rico  
*Associate Professor of Chemistry and Head of Department of Chemistry and Nutrition, School of Tropical Medicine of the University of Puerto Rico under the Auspices of Columbia University* (2, 1944)
- Ashburn, Llewellyn L, M D National Institute of Health, Bethesda 14, Md *Senior Surgeon, U S Public Health Service* (4, 1947)
- Ashby, Winifred M, Ph D 305 10th St, N E, Washington, D C *Senior Scientist, Federal Security Agency (St Elizabeth's Hospital)* (6, 1923)
- Ashman, Richard, M S, Ph D School of Medicine, Louisiana State University, New Orleans  
*Professor of Physiology* (1, 1925)
- Astwood, Edwin Bennet, M D, C M, Ph D Pratt Diagnostic Hospital, 30 Bennet St, Boston, Mass *Research Professor of Medicine at Tufts Medical School* (1, 1939)
- Atkin, Lawrence, Ph D Wallerstein Labs, 180 Madison Ave, New York 16, N Y *Research Chemist* (2, 1946, 5, 1946)
- Aub, Joseph C, M D Harvard Medical School, Boston 15, Mass *Professor of Research Medicine* (1, 1919, 5, 1933)
- Austin, J Harold, M D 711 Maloney Clinic, 36th and Spruce Sts, Philadelphia, Pa *Director, Pepper Laboratory* (2, 1922)
- Avery, O T, M D, Sc D, LL D Hoods Hill Road, Nashville, Tenn *Member Emeritus, Rockefeller Institute for Medical Research* (4, 1921, 6, 1920)
- Axelrod, Bernard, Ph D Western Regional Research Laboratory, Albany, Calif *Associate Chemist* (2, 1948)
- Axtmayer, Joseph H B S A M, Ph D University of Puerto Rico Rio Piedras, Puerto Rico  
*Professor of Chemistry* (5 1935)
- Ayer, Conrad, M D Bureau of Health Augusta, Maine (6, 1944)
- Bach, L M N, Ph D Tulane Univ School of Med, New Orleans La *Professor of Physiology* (1, 1945)
- Bachem, Albert, Ph D College of Medicine, University of Illinois, 1853 W Polk St, Chicago  
*Professor of Biophysics* (1, 1933)
- Bachman, Carl, M D University of Pennsylvania School of Medicine, Philadelphia *Prof of Obstetrics and Gynecology* (2, 1941)
- Baer, Erich, Ph D Banting and Best Department of Medical Research, 100 College St, Toronto, Ontario, Canada *Associate Professor* (2, 1942)
- Baernstein, Harry D, M S, Ph D National Institute of Health, Bethesda, Md *Senior Biochemist* (2, 1934)
- Baetjer, Anna M, D Sc Johns Hopkins School of Hygiene and Public Health, 615 N Wolfe St, Baltimore 5, Md *Assistant Professor of Physiological Hygiene* (1, 1929)
- Bahrs, Alice M, M A, Ph D Deaconess Hospital, Spokane, Wash (1, 1933)
- Bailey, Cameron Vernon, M D, C M 303 E 20th St, New York City *Clinical Professor of Medicine, New York Post-Graduate Medical School, Columbia University* (2, 1920, 5, 1933)
- Bailey, Orville T M D Harvard Univ Medical School, 25 Shattuck St, Boston, Mass *Assistant Professor in Pathology* (4, 1939)
- Bailey, Percival, M D, Ph D Univ of Illinois College of Medicine, 1853 West Polk St, Chicago 12, Ill *Professor of Neurology and Neurosurgery* (1, 1941)
- Baitsell, George Alfred, A M, Ph D Yale University, Osborn Zoological Laby, 165 Prospect St, New Haven, Conn *Professor of Biology* (1, 1915)
- Baker, A B, M D University of Minnesota Medical School, 19 Millard Hall, Minneapolis  
*Director and Professor, Division of Neurology and Neuropathology* (4, 1940)
- Baker, James A, M D New York State Veterinary College, Cornell University, Ithaca, N Y  
*Professor of Bacteriology* (4, 1947)
- Baker, Roger D, M D Medical College of Alabama, Birmingham 5 *Professor of Pathology* (4, 1939)
- Baldes, Edward J, A M, Ph D 427 Fifth Ave, S W, Rochester, Minn *Assistant Professor of Physics, Mayo Foundation, Graduate School, University of Minnesota* (1, 1930)
- Baldwin, Francis Marsh, A M, Ph D University of Southern California, Los Angeles *Professor of Zoology and Director of Experimental Marine Biology* (1, 1919)
- Bale, Wilham F, Ph D University of Rochester, School of Medicine and Dentistry, Rochester, N Y *Associate in Radiology* (1, 1943)
- Ball, Eric G, M A, Ph D Harvard Medical School, Boston, Mass *Professor of Biochemistry* (2, 1934)
- Ball, Howard A, M D San Diego County General Hospital, N Front St, San Diego, Calif *Path-*

- ologist, *San Diego County General and Paradise Valley Hospitals* (4, 1937)
- Balls, Arnold Kent**, Ph D Enzyme Research Laboratory, U S Bureau of Agricultural and Industrial Chemistry, Western Regional Research Laboratory, 800 Buchanan St, Albany 6, Calif *Head Chemist, Adjunct Professor, The George Washington University* (on leave) (2, 1932)
- Bang, Frederick B**, M D Johns Hopkins Hospital, Baltimore, Maryland *Assistant Professor in Medicine* (4, 1947)
- Banus, Mario Garcia**, M Sc, D Sc Bright Meadows, Chestertown, Md (1, 1927)
- Bard, Philip, A M**, Ph D Johns Hopkins University School of Medicine, 710 N Washington St, Baltimore, Md *Professor of Physiology, Member National Academy of Sciences* (1, 1929)
- Barker, H A**, Ph D 3048 Life Science Bldg, Univ of California, Berkeley 4, Calif *Professor of Soil Microbiology* (2, 1946)
- Barker, S B**, Ph D College of Medicine, State University of Iowa, Iowa City *Associate Professor of Physiology* (1, 1938)
- Barlow, O W**, M D, Ph D R F D 3 Warwick Road, Winchester, N H (1, 1936, 3, 1944)
- Barnes, B O**, M A, Ph D Box 967, Station Hospital, KAAF, Kingman, Ariz *Professor of Health Education, University of Denver* (1, 1932)
- Barnes, LaVerne A**, B S, M S, Ph D Naval Medical Research Institute, National Naval Medical Center, Bethesda 14, Maryland *Head, Bacteriology Facility* (6, 1931)
- Barnes, Richard Henry**, Ph D Sharp & Dohme, Glenolden, Pa *Director of Biochemical Research, Medical Research Division* (2, 1941, 5, 1944)
- Barnes, Thomas C**, D Sc Hahnemann Medical College and Hospital of Philadelphia, Philadelphia, Pa *Assoc Professor of Pharmacology* (1, 1942, 3, 1948)
- Barnum, Cyrus P, Jr**, Ph D 210 Millard Hall, Univ of Minnesota, Minneapolis 14, Minn *Associate Professor of Physiological Chemistry* (2, 1946)
- Barott, Herbert G**, E E U S Department of Agriculture, National Agricultural Research Center, Beltsville, Md *Biophysicist, Animal Nutrition Division, Bureau of Animal Industry* (5, 1938)
- Barrera, S Eugene**, M D Albany Medical College, New Scotland Ave, Albany, N Y (1, 1937)
- Barron, Donald H**, M S, Ph D, M A (Cambridge) Yale University School of Medicine, New Haven, Conn *Associate Professor of Physiology* (1, 1943)
- Barron, E S Guzman**, M D Dept of Medicine, Univ of Chicago, Chicago 37, Ill *Associate Professor of Biochemistry* (2, 1931)
- Bartley, S Howard**, Ph D P O Box 763, East Lansing, Mich (1, 1935)
- Bass, Allan D**, M S, M D Univ of Syracuse School of Medicine, Syracuse, N Y *Professor of Pharmacology* (3, 1941)
- Batchelder, Esther L**, M A, Ph D 8433 Woodcliff Ct, Silver Spring, Md (5, 1933)
- Bateman, John B**, Ph D Physical & Chem Division, Camp Detrick, Frederick, Md (1, 1945)
- Bates, Robert W**, Ph D E R Squibb and Sons, Biological Laboratories, New Brunswick, N J *Head, Endocrine Development Dept* (2, 1936)
- Batterman, Robert C**, M D New York University College of Medicine, 477 First Ave, New York City *Instructor in Therapeutics* (3, 1941)
- Baudisch, Oskar**, Ph D Saratoga Springs, N Y *Director of Research, Saratoga Springs Authority, State of New York* (2, 1931)
- Bauer, J H**, M D The Rockefeller Foundation, 20 Rue de la Baume, Paris, (8<sup>e</sup>) France (4, 1935)
- Bauer, Walter**, M D Massachusetts General Hospital, Boston *Associate Professor and Tutor in Medicine, Harvard Medical School, Colonel, MC, Army Service Forces Hq 8th Service Command, Dallas, Texas* (1, 1929)
- Baucrnfeind, J C**, M S, Ph D Hoffmann-La Roche, Inc, Nutley 10, N J *Chief of Applied Nutrition* (5, 1947)
- Bauman, Louis**, M D Columbia Presbyterian Medical Center, New York City *Assistant Professor of Clinical Medicine (Retired), Columbia University* (2, 1912)
- Baumann, Carl A**, M S, Ph D Biochemistry Dept, University of Wisconsin, Madison *Professor of Biochemistry* (2, 1938, 5, 1938)
- Baumann, Emil J**, Ph D 7 Church Lane, Scarsdale, N Y *Chemist, Montefiore Hospital* (2, 1922)
- Baumberger, J Percy**, M S, Sc D Physiology Department, Stanford University, Calif *Professor of Physiology* (1, 1921)
- Baxter, James G**, Ph D 228 Sagamore Drive, Rochester 12, N Y *Supervisor, Organic Research Dept, Distillation Products, Inc* (2, 1946)
- Baxter, James H**, M D Dept of Medicine, Johns Hopkins Hospital, Baltimore, Md (3, 1948)
- Bayne-Jones, Stanhope**, M A, Sc D, M D New York Hospital, Cornell Medical Center, 525 E 68th St, New York 21, N Y *President, Joint Administrative Board* (4, 1927, 6, 1917)
- Bazett, Henry C**, M A, M D, F R C S University of Pennsylvania, School of Medicine, Philadelphia *Professor of Physiology* (1, 1921)
- Beach, Eliot F**, Ph D Metropolitan Life In-

- suance Co, New York City *Research Biochemist* (2, 1941, 5, 1942)
- Beadle, Buell W**, M S, Ph D George W Gooch Lab, Ltd, Los Angeles, Calif *Director* (2, 1947)
- Bean, John W**, M S, Ph D, M D University of Michigan, Ann Arbor *Professor of Physiology* (1, 1932)
- Beard, Howard H**, Ph D Holy Cross Hospital, Chicago, Ill (2, 1928, 5, 1933)
- Beard, Joseph W**, M D Duke Hospital, Durham, N C *Professor of Surgery* (4, 1938, 6, 1940)
- Beazell, James Myler**, Ph D, M D 104 South Michigan Ave, Chicago, Ill *Instructor in Physiology and Pharmacology, Northwestern Univ School of Medicine* (1, 1939)
- Beck, Claude S**, M D Lakeside Hospital, Cleveland, O *Professor of Neurosurgery, Western Reserve University, Associate Surgeon, Lakeside Hospital* (4, 1930)
- Beck, Lyle V**, M S, Ph D 9503 Edgley Road Bethesda, Md (1, 1941)
- Becker, R Frederick**, M S, Ph D Dept of Anatomy, Univ of Washington, Seattle 5, Wash (1, 1941)
- Becker, Theodore J**, M A, Ph D Sterling Winthrop Research Institute, 33 Riverside Avenue, Rensselaer, N Y *Head, Pharmacology Section* (3, 1944)
- Beckman, Harry**, M D Marquette University School of Medicine, Milwaukee, Wis *Professor and Director of the Department of Pharmacology* (3, 1937)
- Beecher, Henry K**, M D Massachusetts General Hospital, Boston *Dorr Professor of Research in Anaesthesia, Harvard Medical School, Anesthetist-in Chief, Massachusetts General Hospital* (3, 1940)
- Behnke, Albert R**, M S, M D Naval Medical Research Institute, Bethesda, Md *Executive Director* (1, 1946)
- Behre, Jeanette Allen**, Ph D Department of Biochemistry, College of Physicians and Surgeons, 630 W 168th St, New York City *Associate* (2, 1925)
- Behrmann, Vivian G**, Ph D Wayne Univ, Detroit, Mich *Instr Grad School, Henry Ford Hosp, Res Physiologist* (1, 1948)
- Belding, David L**, M D Boston University School of Medicine, Boston, Mass *Professor of Bacteriology and Experimental Pathology* (4, 1927)
- Belding, Harwood S**, Ph D QMC Climatic Laboratory, Lawrence, Mass *Director* (1, 1945)
- Bell, E T**, M D 110 Anatomy Bldg, University of Minnesota, Minneapolis *Professor of Pathology* (4, 1931)
- Bender, M B**, M D New York University College of Medicine *Associate Professor of Neurology and Head of the Laboratory of Experimental Medicine* (1, 1947)
- Benedict, Francis Gano**, Ph D, Sc D, M D Machiasport, Me *Member of the National Academy of Sciences* (1R, 1904, 2, 1906)
- Benditt, Earl P**, M D Department of Pathology, University of Chicago Clinics, Chicago 37, Ill *Assistant Professor* (4, 1947)
- Benham, Olive Ray**, BS Connecticut State Department of Health, Bureau of Laboratories, Hartford *Chief Serologist* (6, 1944)
- Bennett, A Lawrence**, Ph D, M D College of Medicine, University of Nebraska, Omaha *Professor of Physiology and Pharmacology* (1, 1941)
- Bennett, Granville A**, M D University of Illinois College of Medicine, 1853 West Polk Street, Chicago *Professor of Pathology* (4, 1931)
- Bennett, Henry S**, M D University of Washington School of Medicine, Seattle, Wash *Dept of Anatomy* (1, 1946)
- Bennett, Leslie L**, M D University of California, Berkeley 4 *Assistant Professor of Physiology* (1, 1945)
- Bennett, Mary Adelia**, M A, Ph D Lankenau Hospital Research Institute, Philadelphia, Pa *Research Biochemist* (2, 1941)
- Benson, Clara C**, Ph D 160 Dorset St, West, Port Hope, Ontario, Canada *Professor Emeritus of Food Chemistry, University of Toronto* (2, 1906)
- Berg, Benjamin N**, M D 630 W 168th St, New York City *Associate in Pathology, Columbia University, College of Physicians and Surgeons* (4, 1928)
- Berg, Clarence P**, Ph D Chemistry Department, State University of Iowa, Iowa City *Professor of Biochemistry* (2, 1933, 5, 1936)
- Berg, William N**, Ph D 225 W 106th St, New York City *Biochemist* (2, 1906)
- Bergeim, Olaf**, M S, Ph D Univ of Illinois College of Medicine, Chicago *Prof of Biochemistry* (1, 1916, 2, 1914)
- Bergmann, Werner**, Ph D Sterling Chemistry Lab, Yale University, New Haven, Conn *Professor of Chemistry* (2, 1934)
- Berkson, Joseph**, M A, M D, D Sc Mayo Clinic, Rochester, Minn (1, 1933)
- Bernard, Richard**, M Sc, Ph D Department of Biology, Laval University, Blvd de l'Entente, Quebec, Canada *Assistant Professor of Physiology* (1, 1947)
- Bernheim, Frederick**, Ph D Box 3109, Duke Medical School, Durham, N C *Professor of Pharmacology* (2, 1933, 3, 1935)
- Bernthal, Theodore G**, M S, M D Dept of Physiology, Medical College, State of South

- Carolina, Charleston 16, S C *Professor of Physiology* (1, 1932)
- Berry, George Packer, M D University of Rochester, Rochester, N Y *Associate Dean, Professor of Bacteriology, Associate Professor of Medicine* (4, 1938, 6, 1934)
- Bessey, Otto A, Ph D University of Illinois College of Medicine, 1853 W Polk St, Chicago, Ill *Professor and Head, Dept of Biological Chemistry* (2, 1938, 5, 1913)
- Best, Charles Herbert, C B E, M A, M D, D Sc (London), D Sc (Chicago), F R S, F R C P (c), University of Toronto, Toronto, Ont, Canada *Director, Banting and Best Department of Medical Research and Department of Physiology* (1, 1923, 2, 1923)
- Bethell, Frank H, M D 409 Lenawee Drive, Ann Arbor, Mich *Professor of Internal Medicine and Assistant Director of the Thomas Henry Simpson Memorial Institute* (4, 1936)
- Bethke, Roland M, M S, Ph D Ohio Agricultural Experiment Station, Wooster *In Charge of Nutritional Investigations* (2, 1928, 5, 1933)
- Beutner, R, M D, Ph D 235 N 15th St, Philadelphia, Pa *Professor and Head of Department of Pharmacology, Hahnemann Medical College* (1, 1924, 3, 1924)
- Beyer, Karl H, Ph D, M D Medical-Research Division, Sharp and Dohme, Inc, P O Box 7259, Glenolden, Pa *Director of Pharmacological Research* (1, 1942, 3, 1944)
- Bier, Otto, M D Instituto Postal 119 \, Sao Paulo, Brazil (6, 1947)
- Bieter, Raymond N, M D, Ph D University of Minnesota, Minneapolis *Professor of Pharmacology* (3, 1930)
- Bills, Charles E, M A, Ph D Mead Johnson & Co, Evansville, Ind *Director of Research* (2, 1928, 5, 1935)
- Bing, Franklin C, Ph D 1135 Fullerton Ave, Chicago, Ill *Director, American Institute of Baking, Assistant Professor of Physiology, Northwestern University Medical School* (2, 1931, 5, 1934)
- Bing, Richard J, M D Department of Surgery, Johns Hopkins Hospital, Baltimore 5, Md *Assistant Professor of Surgery* (1, 1942)
- Binger, Carl A, M D 125 E 73rd St, New York City *Assistant Professor of Clinical Medicine (Psychiatry), Cornell University Medical College* (1, 1927)
- Binkley, Francis, Ph D School of Medicine, University of Utah, Salt Lake City, Utah *Associate Professor* (2, 1947)
- Binkley, Stephen Bennett, M S, Ph D Bristol Laboratories, Inc, Syracuse, N Y *Assistant Director of Research* (2, 1941)
- Bird, Herbert R, M S, Ph D Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md *Senior Biochemist* (5, 1947)
- Bird, Orson D, M S, Ph D Research Laboratories, Parke, Davis and Co, Detroit 32, Mich *Research Biochemist* (2, 1947)
- Bisbey, Bertha, A M, Ph D Gwynn Hall, University of Missouri, Columbia *Professor of Nutrition* (5, 1933)
- Bischoff, Fritz E, M S, Ph D Cottage Hospital, Santa Barbara, Calif *Director of Research* (2, 1928, 5, 1933)
- Bishop, George H, Ph D Washington University Medical School, Euclid and Kingshighway, St Louis, Mo *Professor of Bio-Physics* (1, 1923)
- Biskind, Gerson R, M D 240 Stockton St, San Francisco, Calif *Pathologist, Mt Zion Hospital, Clinical Instructor in Pathology, University of California Medical School* (4, 1944)
- Black, Alex, M S, Ph D Department of Animal Nutrition, Pennsylvania State College, State College, Penn *Professor of Animal Nutrition* (5, 1917)
- Black, Edgar C, Ph D Department of Biology and Botany, University of British Columbia, Vancouver, B C, Canada (1, 1943)
- Black, Simon, Ph D Univ of Chicago, Chicago, Ill *Instructor, Dept of Medicine* (2, 1948)
- Blair, Edgar A, M S, Ph D Armored Medical Research Laboratory, Fort Knox, Ky *Lt Col* (1, 1936)
- Blair, Henry A, M Sc, Ph D University of Rochester School of Medicine and Dentistry, Rochester, N Y *Professor of Physiology and Director of Dept of Radiation Biology* (1, 1931)
- Blake, Francis G, M D, M A (hon), Sc D Yale University School of Medicine, New Haven, Conn *Sterling Professor of Medicine* (4, prior to 1920, 6, 1921)
- Blanchard Ernest W, Ph B, M S, Ph D Schieffelin and Co, 30 Cooper Sq, New York 3, N Y *Director of Research* (1, 1946)
- Blankenhorn, M A, M D University of Cincinnati, Cincinnati, O *Professor of Medicine* (4, 1932)
- Blatherwick, Norman R, M S, Ph D, Sc D Metropolitan Life Ins Co, 1 Madison Ave, New York City *Director of Biochemical Laboratory* (1, 1915, 2, 1915, 5, 1934)
- Blau, Nathan F, Ph D Veterans Administration Hospital, Wichita, Kan *Research Biochemist* (2, 1928)
- Blish, Morris J, M A, Ph D Amino Products Company, Rossford, O *Research Director* (2, 1944)
- Bliss, Alfred, M A, Ph D Tufts College Medical School, Boston, Mass *Associate Professor of Physiology* (1, 1947)
- Bliss, Chester Ittner, Ph D Conn Agr Expt Sta, P O Box 1106, New Haven *Biometrician*,



- Lecturer in Biometry, Yale University* (3, 1944)
- Bliss, Eleanor A**, Sc D Department of Preventive Medicine, Johns Hopkins Hospital, 615 N Wolfe St, Baltimore, Md *Associate in Preventive Medicine, Johns Hopkins University, School of Medicine* (6, 1931)
- Bloch, Konrad**, Ph D Department of Biochemistry, University of Chicago, Chicago, Illinois *Associate Professor of Biochemistry* (2, 1944)
- Block, Richard J**, Ph D 15 Cooper Rd, Scarsdale, N Y *Director of Research, C M Armstrong Co, Associate, Department of Physiology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospital* (2, 1934, 5, 1933)
- Block, Walter D**, M S, Ph D 813 East McCreight Ave, Springfield, Ohio (2, 1942)
- Bloom, William**, M D 1419 E 56th St, Chicago, Ill *Professor of Anatomy, University of Chicago* (4, 1930)
- Bloomfield, A L**, M D Stanford University Hospital, San Francisco, Calif *Professor of Medicine* (3, 1927, 4, 1927)
- Bloor, W R** A M, Ph D, LL D School of Medicine and Dentistry, University of Rochester, Rochester, N Y *Professor of Biochemistry* (1R, 1915, 2, 1910)
- Blum, Harold F**, Ph D Department of Biology, Princeton University, Princeton, N J *Physiologist, National Cancer Institute, and Visiting Lecturer* (1, 1923)
- Blumberg, Harold**, Sc D Research Laboratories, Endo Products, Inc 84 40 101st St, Richmond Hill 18, N Y (5, 1942)
- Blumenstock, Julius**, M D Veterans Administration Hospital, Sheridan, Wyoming (1, 1925)
- Blumgart, Hermann L**, M D Beth Israel Hospital, 330 Brookline Ave, Boston, Mass *Associate Professor of Medicine, Harvard Medical School, Lt Col, M C* (1, 1927)
- Blunt, Katharine**, Ph D, LL D 38 Glenwood Ave, New London, Conn *President Emeritus, Connecticut College for Women* (2, 1921)
- Bock, Joseph C**, Ch E, Ph D 2324 N 46th St, Milwaukee 10, Wis *Professor Emeritus of Biochemistry, Marquette Univ Medical School, Biochemist, Milwaukee County Hospital* (2, 1916)
- Bodansky, Aaron**, Ph D Hospital for Joint Diseases, 1919 Madison Ave, New York City *Biological Chemist* (2, 1926)
- Bodansky, Oscar**, Ph D, M D Memorial Hospital, Cancer Center, New York City *Clinical Biochemist Assoc Member, Sloan Kettering Institute for Cancer Research Assoc Professor of Clinical Pharmacology Cornell Univ Medical College* (2, 1937, 3, 1942)
- Bodine, Joseph Hall**, Ph D State University of Iowa, Iowa City *Professor and Head of Department of Zoology* (1, 1925)
- Boell, Edgar J**, Ph D Osborn Zoological Laboratory, Yale University, New Haven, Conn *Ross G Harrison Professor of Experimental Zoology* (1, 1942)
- Boger, William P**, M D Medical Research Division, Sharp and Dohme, Inc Glenolden, Pa *Assoc Medical Director, Instructor in Medicine, Univ of Pa Sch of Medicine and Graduate Sch, of Medicine* (3, 1948)
- Bogert, L Jean**, Ph D Hotel Claremont, Berkeley, Calif (2, 1917)
- Bogert, Marston Taylor**, Sc D, LL D, R N D 1158 Fifth Ave, Apt 14B, New York 29, N Y *Professor Emeritus of Organic Chemistry, Columbia University, Member, National Academy of Sciences* (2, 1925)
- Boivin, Andre** M D La Faculte de Medecine de Strasbourg, Strasbourg, France *Professeur, member de l'Academie de Medecine de France* (6, 1948)
- Bolliger, Adolph**, Ph D Gordon Craig Research Laboratories, University of Sydney, Sydney, Australia *Director of Research* (2, 1928)
- Bollman, J L**, M D Mayo Clinic, Rochester, Minn *Chairman, Division of Experimental Medicine, Professor of Physiology, Mayo Foundation* (4, 1927)
- Bond, Glenn C**, Ph D, M D The Upjohn Co, Research Laboratories, Kalamazoo, Mich *Assistant Dept Head, Bacteriology Research* (6, 1939)
- Bondi, Amedeo** Hahnemann Medical College, Philadelphia, Pa *Professor and Head, Dept of Bacteriology* (6, 1948)
- Bonner, David M**, Ph D Osborn Botanical Laboratory, Yale Univ, New Haven, Conn *Assoc Professor and Research Associate* (2, 1948)
- Bonnycastle, Desmond D**, M D, Ph D Yale University School of Medicine, 333 Cedar Street, New Haven, Conn *Assistant Professor of Pharmacology* (3, 1947)
- Bonsnes, Roy W**, B S, Ph D Department of Biochemistry, Cornell University Medical College, 1300 York Ave, New York 21, N Y *Assistant Professor of Biochemistry in Obstetrics* (2, 1947)
- Booher, Lela E**, Ph D General Mills, Inc, Minneapolis Minn *Chief Nutritionist and Director of Nutrition Lab* (2, 1933, 5, 1933)
- Booker, Walter M**, Ph D Howard Univ Sch of Med, Washington, D C *Assoc Professor of Pharmacology* (1, 3, 1948)
- Bookman, Samuel**, M A, Ph D 624 Madison Ave, New York City *Consulting Chemist, Mt Sinai Hospital* (2, 1912)
- Boor, Alden K**, M S, Ph D Basic Science Division, Camp Detrick, Frederick, Md (2, 1931)
- Boothby, Walter M**, M D Mayo Clinic Rochester, Minn *Emeritus Chief, Clin Metabolism*

- Sect, Div of Med, Eminent Professor of Exper Metabolism, Mayo Found Univ of Lund, Sweden Special Res Consultant, Dept of Physiology (1, 1915, 2, 1920, 3R, 1923, 1, 1924)*
- Bordley, James, III, M D** Mary Imogene Bassett Hospital, Cooperstown, N Y (1, 1938)
- Borek, Ernest, Ph D** College of the City of New York, Convent Ave and 140th St, New York City Asst Professor, Research Associate, Biochemistry, Columbia Univ (2, 1947)
- Boroff, Daniel A, M A, M S** Rheumatic Fever Research Institute, 306 S California Ave, Chicago, Ill Medical Bacteriologist, Project Chief (6, 1947)
- Borsook, Henry, M D, Ph D** California Institute of Technology, Pasadena 4 Professor of Biochemistry (2, 1931)
- Bosshardt, David K, M S, Ph D** Medical Research Division, Sharp and Dohme, Inc, Glenolden, Pennsylvania Research Biochemist (5, 1947)
- Bosworth, Alfred Willson, A M, M D R D 4,** Circleville, O Consulting Chemist (2, 1936, 5, 1935)
- Bott, Phyllis A, M S, Ph D** Woman's Medical College of Pennsylvania, Henry Ave and Abbot'sford Rd, Philadelphia Professor of Physiological Chemistry and Chairman of the Department (2, 1938)
- Boucher, Robert V, M A, Ph D** 303 Frear Labs State College, Pa Professor of Agricultural and Biological Chemistry (5, 1945)
- Bouman, H D, M D** University of Wisconsin Medical School, Madison Professor of Physical Medicine (1, 1943)
- Bourne, Wesley, M D, C M, M Sc, F R C P, D A (R C P & S, Eng), F A C A** McGill University, Montreal, Canada Chairman, Dept of Anesthesia (3, 1936)
- Bourquin, Helen, M S, Ph D** 1331 N Tejon St, Colorado Springs, Colo (1, 1925)
- Bowen, William J, Ph D** USPHS, National Institutes of Health, Bethesda, Md Sr Asst Scientist (1, 1948)
- Bowman, Donald E, A M, Ph D** 6956 Warwick Rd, Indianapolis, Ind Associate Professor of Biochemistry, Indiana University School of Medicine (2, 1944)
- Bowman, Katherine L, B A** 20 Plaza Street, Brooklyn 17, N Y (6, 1946)
- Boxer, George E, Ph D** 605 Girard Ave, Westfield, N J Senior Chemist, Research and Development Division, Merck & Co, Inc (2, 1916)
- Boyd, Eldon M, M A, M D, C M** Queen's University, Kingston, Ontario, Canada Professor and Head of the Department of Pharmacology (3, 1941)
- Boyd, J Milford, M S, Ph D** Hahnemann Medical Coll, Philadelphia, Pa Prof and Head, Dept of Chemistry (2, 1947)
- Boyd, T E, Ph D** 9 Walworth Ave, Scarsdale, N Y (1, 1924)
- Boyd, William C, A M, Ph D** Boston University School of Medicine, 80 E Concord St, Boston, Mass Associate Professor of Biochemistry (6, 1933)
- Boyden, Allan A, Rutgers Univ, New Brunswick, N J** Professor of Zoology (6, 1918)
- Boyden, Edward A, A M, Ph D** University of Minnesota, Minneapolis 14 Professor of Anatomy and Chairman of the Department (1, 1929)
- Boyer, Paul D, M S, Ph D** Division of Biochemistry, College of Agriculture, University of Minnesota, St Paul 1 Associate Professor (2, 1911)
- Boyle, Paul E, D M D** School of Dentistry, University of Pennsylvania, 40th and Spruce Sts, Philadelphia 1 Professor of Oral Pathology (1, 1939)
- Bozicevich, John, M A** USPHS, National Institutes of Health, Bethesda, Md Head, Subsection of Immunology, Trop Dis Div (6, 1918)
- Bozler, Emil, Ph D** Ohio State University, Columbus Professor of Physiology (1, 1932)
- Bradbury, James T, M S, Sc D** Dept of Obstetrics and Gynecology, University Hospitals, Iowa City Assistant Professor of Obstetrics and Gynecology (1, 1941)
- Bradley, Harold C, Ph D** 2639 Durant Ave, Berkeley, Calif (1, 1911, 2, 1908)
- Bradley, Stanley E, M D** College of Physicians and Surgeons, 620 West 168th Street, New York 32, N Y Assistant Professor (1, 1947)
- Bradley, William B, Ph D** American Institute of Baking, 1046 Elmwood Ave, Wilmette, Ill Director of Laboratories (1, 1939)
- Branch, Charles F, M D** The American College of Surgeons, 40 L Erie St, Chicago 11, Ill Assistant Director (1, 1940)
- Branch, E Arnold G, M D** Lancaster Hospital, St John, N B, Canada Director of Laboratories (4, 1929)
- Brand, Erwin, Ph D** 630 W 168th St, New York City Associate Professor of Biological Chemistry, Columbia University (2, 1929)
- Brandes, W W, M D** Roosevelt Hospital, W 59th St, New York City (4, 1931)
- Branham, Sara E, Ph D, M D, Sc D** National Institute of Health, Bethesda, Md Senior Bacteriologist (6, 1926)
- Branion, Hugh Douglas, M A, Ph D** Ontario Agricultural College, Guelph, Canada Professor and Head of Dept of Animal Nutrition (5, 1933)
- Brassfield, Charles R, Ph D** University of Michigan, Ann Arbor Associate Professor of Physiology (1, 1937)
- Bratton, Andrew Calvin, Jr, M D, Ph D** Research Laboratories, Parke, Davis and Co,

- Detroit 32, Mich *Director of Pharmacological Research* (3, 1941)
- Brauer, Ralph W, Ph D Louisiana State Univ School of Medicine, New Orleans, La *1st Professor of Pharmacology* (3, 1948)
- Braun, Herbert A, Ph D Food & Drug Administration, Federal Security Agency, Washington, D C *Associate Pharmacologist* (3, 1941)
- Brazier, Mary A B, Ph D Electroencephalographic Laboratory, Massachusetts General Hospital, Boston 14 *Research Associate in Neuropathology*, Harvard Medical School (1, 1947)
- Breidts, Charles, M D Univ of Pennsylvania School of Medicine, Philadelphia, Pa *Instructor in Pathology* (4, 1948)
- Brewer, Carl R, Ph D Camp Detrick Frederick, Md *Chief, Bacterial Nutrition Branch Biological Div, Chemical Corps* (2, 1948)
- Brewer, George, M D 1665 Lamont St N W, Washington, D C (1, 1937)
- Brewer, John H, Ph D Hynson Westcott and Dunning, Baltimore, Md *Director of Biological Research* (6, 1948)
- Brewer, Nathan R, Ph D Univ of Chicago Chicago, Ill *Lecturer in Physiology* (1, 1948)
- Bridge, Edward M, M D University of Buffalo Medical School, Buffalo N Y *Research Professor, Department of Pediatrics* (2, 1940)
- Briggs, A P, M D University of Georgia Medical College, Augusta *Professor of Biochemistry* (2, 1923)
- Briggs, David, R, M S, Ph D Division of Agricultural Biochemistry, University Farm, University of Minnesota, St Paul 8, Minn *Professor of Agricultural Biochemistry, Chemist, Minn Agriculture Experiment Station* (2, 1946)
- Briggs, George M, M S, Ph D University of Minnesota, University Farm, St Paul, Minn *1st Professor, Poultry Nutrition* (5, 1947)
- Brink, Frank, Jr, Ph D Johnson Research Foundation, University of Pennsylvania, Philadelphia *Fellow in Medical Physics, Johnson Research Foundation, Lecturer in Biophysics, Graduate School, University of Pennsylvania* (1, 1942)
- Brinkhous, K M, M D Dept of Pathology, University of North Carolina School of Medicine, Chapel Hill, N C *Professor of Pathology* (4, 1939)
- Britton, Sydney W, M D University of Virginia School of Medicine, Charlottesville, Va *Professor of Physiology* (1, 1925)
- Brobeck, John R, M D, Ph D Yale University School of Medicine, New Haven Conn *Assistant Professor of Physiology* (1, 1943)
- Brodie, Bernard B, Ph D New York University Research Service, Goldwater Memorial Hospital, New York City *Research Associate in Biochemistry, Assistant Professor of Pharmacology, New York University Medical College* (2, 1940, 3, 1945)
- Brody, Samuel, M A, Ph D Dairy Building, University of Missouri, Columbia *Professor of Dairy Husbandry, College of Agriculture and Agricultural Experimentation* (2, 1929, 5, 1933)
- Broh-Kahn, Robert H, M D May Institute for Med Res, Cincinnati, Ohio *Asst Director* (1, 1948)
- Brönfenbrenner, J J, Ph D, D P H Washington University School of Medicine, St Louis, Mo *Professor of Bacteriology and Immunology* (4, 1940, 6, 1918)
- Bronk, Detlev W, Ph D, Sc D Johns Hopkins University, Baltimore, Md *President Director, Eldridge Reeves Johnson Found for Med Physics Member, Natl Acad of Sci Chairman, Natl Research Council* (1, 1927)
- Brookes, Margaret C Hessler, A M, Ph D University of Chicago, Chicago, Ill *Assistant Professor, Department of Home Economics* (5, 1935)
- Brookhart, John M, B A, M S, Ph D 1940 W Eddy St, Chicago 13, Illinois *Assistant Professor Physiology, Loyola Univ* (1, 1946)
- Brooks, Chandler McCuskey, M A, Ph D The Long Island College of Medicine, Dept of Physiology and Pharmacology, Brooklyn, N Y (1, 1934)
- Brooks, Clyde, Ph D, M D, LL D University Clinic, 2506 Ponce de Leon Blvd Coral Gables, Fla (1, 1910, 3, 1912)
- Brooks, Matilda Moldenhauer, M S, Ph D Department of Zoology, University of California, Berkeley *Research Associate in Biology* (1, 1923)
- Brooks, Sumner Cushing, Ph D University of California, Berkeley *Professor of Zoology* (1, 1923)
- Broun, Goronwy Owen, M D 1325 S Grand Blvd, St Louis, Mo *Professor of Internal Medicine, St Louis University* (4, 1927)
- Brown, Claude P, M D 1930 Chestnut St, Philadelphia, Pa (6, 1913)
- Brown, Dugald E S M A, Ph D Bermuda Biological Station, St George's W Bermuda (1, 1932)
- Brown Ethan Allan, L R C P (Eng), A R C S (London), 75 Bay State Rd, Boston, Mass *Lecturer in Medicine, Tufts College Medical School, Physician in chief, Allergy Clinic, Boston Dispensary* (6, 1946)
- Brown, Frank A, Jr, M A, Ph D Zoological Laboratories, Northwestern University, Evanston, Ill *Associate Professor of Zoology* (1, 1940)
- Brown, George B, M S, Ph D Sloan-Kettering Inst for Cancer Res, New York City *Member, Cornell Univ Med Coll, 1st Prof of Biochemistry* (2, 1947)

- Carmichael, Leonard**, Ph D , Sc D , Litt D , LL D Tufts College, Medford, Mass *Director, the Tufts College Research Laboratory of Sensory Psychology and Physiology and President of the College* (1, 1937)
- Carpenter, Thorne M.**, Ph D Sc D (hon ) 27 Market St , Foxboro, Mass (1R, 1915, 2, 1909, 5, 1935)
- Carr, C Jelleff**, Ph D School of Medicine, University of Maryland, Baltimore *Associate Professor of Pharmacology* (3, 1940)
- Carr, Jesse L.**, M D University of California Medical School, Third and Parnassus Aves , San Francisco *Assistant Professor of Pathology* (4, 1940)
- Carruthers, Christopher**, Ph D Barnard Free Skin and Cancer Hospital, St Louis, Mo *Research Associate* (2, 1948)
- Carter, Herbert E.**, M A , Ph D 452 Noyes Laboratory, Urbana, Ill *Professor of Biochemistry, University of Illinois* (2, 1937, 5, 1941)
- Cartland, George F.**, M S , Ph D The Upjohn Co , Research Dept , Kalamazoo, Mich *Head, Antibiotics Research* (2, 1936)
- Cary, Charles A.**, S B Dury Research Laboratory, Beltsville, Md *Chief, Division of Nutrition and Physiology, Bureau of Dairy Industry, U S Department of Agriculture* (2, 1920)
- Casey, Albert Eugene**, M D 1907 Wellington Rd , Birmingham 9, Ala *Pathologist, Baptist Hospital* (4, 1933)
- Cash, James Robert**, M D University Hospital, Charlottesville, Va *Professor of Pathology, University of Virginia* (4, 1924)
- Castle, Edward S.**, M A , Ph D Biological Laboratories, Harvard University, Divinity Ave , Cambridge, Mass *Associate Professor of General Physiology* (1, 1934)
- Castle, William B.**, M D , S M (Hon Yale), M D (Hon Utrecht) Boston City Hospital, Boston, Mass *Professor of Medicine, Harvard Medical School, Director, Thorndike Memorial Laboratory and Director, II and IV Medical Services (Harvard), Boston City Hospital* (4, 1942)
- Catchpole, Hubert Ralph**, Ph D 1853 W Polk St , Chicago 12, Ill *Research Associate in Pathology, University of Chicago College of Medicine* (1, 1941)
- Cathcart, E P.**, M D , D Sc , LL D University of Glasgow, Glasgow, Scotland *Dean of University* (5, 1935)
- Catron, Lloyd**, M D The City Hospital, Akron, O *Pathologist* (4, 1939)
- Cattell, McKeen, A M.**, Ph D , M D Cornell University Medical College, 1300 York Ave , New York City *Professor of Pharmacology* (1, 1923, 3, 1924)
- Cavelti, Philip A.**, M D 1631 31st Ave, San Francisco, Calif (6, 1917)
- Cerecedo, Leopold R.**, Ph D Fordham University, New York City *Professor of Biochemistry* (2, 1931, 5, 1945)
- Chadwick, Feigh Edward**, Ph D Medical Division, Army Chemical Center, Md (1, 1914)
- Chaikoff, I L.**, A M , Ph D , M D University of California, Berkeley *Associate Professor of Physiology* (1, 1932)
- Chalkley, Harold W.**, A M , Ph D U S Public Health Service, National Institute of Health, Bethesda, Md *Senior Physiologist* (1, 1932)
- Chamberlain, Edward**, M D Temple Univ Med Sch , Philadelphia, Pa *Professor of Radiology* (1, 1948)
- Chambers, Alfred H.**, Ph D University of Vermont School of Medicine, Burlington, Vt *Dept of Physiology* (1, 1946)
- Chambers, Leslie Addison**, M S , Ph D Camp Detrick, Frederick, Md *Chief, Physical and Chemical Division* (1, 1940, 6, 1948)
- Chambers, Robert, A M.**, Ph D Marine Biological Laboratory, Woods Hole, Mass *Director of Laboratory of Cellular Physiology, Research Professor Imcritus, New York University* (1, 1932)
- Chambers, William H.**, M S , Ph D Medical Division, Army Chemical Center, Md *Chief, Toxicology Branch* (1, 1924, 5, 1933)
- Chandler, Caroline A.**, M D 615 N Wolfe St , Baltimore 5, Md *Assistant Professor of Preventive Medicine* (6, 1938)
- Chandler, Joseph P.**, M S , Ph D University of Michigan Med Sch , Ann Arbor, Mich *Asst Prof of Biological Chemistry* (2, 1944, 5, 1944)
- Chang, Min Cheuh**, B Sc , Ph D Worcester Foundation, Shrewsbury, Mass *Associate Fellow* (1, 1946)
- Chanutin, Alfred**, Ph D Box 1862 (University Station), Charlottesville, Va *Professor of Biochemistry, University of Virginia* (2, 1925)
- Chapanis, Alphonse**, Ph D Johns Hopkins Univ , Baltimore, Md *Asst Professor of Psychology* (1, 1948)
- Chapman, C W.**, M Sc , Ph D University of Maryland, Baltimore *Professor of Pharmacology* (3, 1932)
- Chargaff, Erwin**, Ph D Columbia University, College of Physicians and Surgeons, 630 W 168th St , New York City *Associate Professor of Biological Chemistry* (2, 1935)
- Charipper, Harry Adolph**, M S , Ph D Washington Square College of Arts and Sciences, 100 Washington Square East, New York City *Professor of Biology and Chairman of the Department* (1, 1941)
- Chase, Aurin M.**, A M , Ph D Department of

- Biology, Princeton University, Princeton, N J  
*Research Associate, Assistant Professor* (1, 1939)
- Chase, Harold F, B S, M D Hartford Hospital, Hartford 15, Conn (3, 1944)
- Chase, Merrill W, M S, Ph D Rockefeller Institute, 66th St and York Ave, New York City *Member of Staff* (6, 1938)
- Chasis, Herbert, M D, Med Sc D 44 E 67th St, New York City *Assistant Professor of Medicine, New York University, College of Medicine* (1, 1941)
- Chatfield, Charlotte, B S Food and Agriculture Organization of the United Nations, Washington, D C *Nutrition officer* (5, 1941)
- Chatterjee, Hernendra Nath, M D Calcutta Univ, Calcutta, India *Teacher, Fellow, Royal Society of Tropical Medicine (Eng)* (6, 1948)
- Cheldelin, Vernon H, M S, Ph D Department of Chemistry, Oregon State College, Corvallis, Ore *Professor of Chemistry* (2, 1947, 5, 1946)
- Chen, Graham, Sc D, M D c/o Parke, Davis and Co, Detroit, Mich (3, 1944)
- Chen, K K, Ph D, M D The Lilly Research Laboratories, Indianapolis, Ind *Director of Pharmacological Research, Lilly Research Laboratories, Professor of Pharmacology, Indiana University School of Medicine, Indianapolis* (1, 1929, 3, 1942)
- Cheney, Ralph H M A, M S, Sc D Biology Dept, Brooklyn College, Bedford Ave and Ave H, Brooklyn 10, N Y (3, 1934)
- Chenoweth, Maynard Burton, M D Dept of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich *Assoc Professor of Pharmacology* (3, 1945)
- Chesney, Alan M, M D The Johns Hopkins Hospital, Baltimore, Md *Dean, Johns Hopkins Medical School, Associate Professor of Medicine* (1, 1925)
- Chow, Bacon F, Ph D Squibb Institute for Medical Research, New Brunswick, N J *Head, Nutritional Development Dept* (2, 1940, 5, 1948, 6, 1944)
- Christensen, Halvor N, M S, Ph D Tufts College Med Sch, Boston, Mass *Prof and Head, Dept of Biochemistry* (2, 1947)
- Christensen, L Royal, Ph D New York University College of Medicine, 477 First Ave, New York City *Asst Professor Dept of Microbiology* (6, 1942)
- Christian, Henry A, M D 20 Chapel St, Brookline, Mass *Hersey Professor of the Theory and Practice of Physics, Emeritus, Harvard University Physician in Chief Emeritus, Peter Bent Brigham Hospital, Boston Visiting Physician, Beth Israel Hospital Boston* (4, 1924)
- Christman, Adam A Ph D University of Michigan Medical School, Ann Arbor *Professor of Biological Chemistry* (2, 1929)
- Chu, Wei-chang, M D 546 West 124th St, Apt 53, New York City (3, 1945)
- Clark, Ada R, M A, Ph D College of Physicians and Surgeons, 630 W 168th St, New York City *Associate, Bacteriology, Teaching and Research* (6, 1936)
- Clark, Byron B, M S, Ph D Tufts College Medical School, 416 Huntington Ave, Boston 15, Mass *Professor of Pharmacology* (3, 1940)
- Clark, Ernest D, A M, Ph D 826 Skinner Bldg, Seattle 1, Wash *Director of the Laboratories, Northwest Branch, National Cannery Association* (2, 1912)
- Clark, George, Ph D Department of Anatomy, Chicago Medical School, 710 S Wolcott Ave, Chicago 12, Ill *Associate Professor of Neuroanatomy* (1, 1943)
- Clark, Guy W, A M, Ph D c/o Lederle Laboratories, Inc, Pearl River, N Y *Technical Director* (2, 1922)
- Clark, Janet Howell, A M, Ph D Anderson Hall, University of Rochester, Rochester, N Y *Dean of the College for Women and Professor in the Division of Biological Sciences* (1, 1922)
- Clark, Paul F, Ph D University of Wisconsin Medical School, Madison *Professor of Medical Microbiology* (4, 1923, 6, 1928)
- Clark, William G, Ph D Scripps Metabolic Clinic La Jolla, Calif (1, 1942)
- Clark, William Mansfield, M A, Ph D, D Sc Johns Hopkins University, Baltimore, Md *Professor of Physiological Chemistry, Member, National Academy of Sciences* (2, 1920)
- Clarke, Hans Thacher, D Sc (London), F I C 630 W 168th St, New York City *Professor of Biological Chemistry, Columbia University, College of Physicians and Surgeons* (2, 1929)
- Clarke, Robert W 6 Audubon Court, Elizabethtown, Kentucky *Physiologist, Armed Med Research Lab, Fort Knox* (1, 1936)
- Clausen, Samuel Wolcott, M D School of Medicine, University of Rochester, Rochester, N Y *Professor of Pediatrics* (2, 1922)
- Cleghorn, Robert Allen, M D D Sc (Aberdeen) Department of Psychiatry, McGill University, Montreal, Quebec, Canada (1, 1937)
- Clowes, George Henry Alexander, Ph D, D Sc (hon), LL D (hon) Eli Lilly & Co, Indianapolis, Ind *Director of Research* (2, 1914, 6, 1919)
- Coburn, Alvin F, M D Rheumatic Fever Research Institute, Chicago, Ill *Director* (6, 1948)
- Code, Charles F, Ph D, M D Mayo Foundation, Rochester, Minn *Professor of Physiology* (1, 1939)

- Coffey, Julia M**, A B Division of Laboratories & Research, New York State Department of Health, Albany, N Y *Associate Bacteriologist* (6, 1937)
- Coghill, Robert D**, M S, Ph D Abbott Laboratories, North Chicago, Illinois *Director of Research* (2, 1932)
- Cohen, Barnett**, M S, Ph D Johns Hopkins University School of Medicine, 710 N Washington St, Baltimore 5, Md *Associate Professor of Physiological Chemistry* (2, 1935)
- Cohen, Milton B**, M D 10616 Euclid Ave, Cleveland, O *Director, The Asthma, Hay Fever and Allergy Foundation* (6, 1931)
- Cohen, Philip P**, Ph D, M D Service Memorial Institute, University of Wisconsin, Madison *Professor of Physiological Chemistry* (2, 1911)
- Cohen, Saul L**, Ph D Univ of Minnesota, Minneapolis, Minn *Asst Professor of Physiological Chemistry* (2, 1948)
- Cohen, Seymour S**, Ph D Children's Hospital, 1840 Bainbridge St, Philadelphia, Pa *Assistant Professor of Physiological Chemistry, University of Pennsylvania School of Medicine* (2, 1946)
- Cohen, Sophia M**, B S Division of Laboratories and Research, New York State Department of Health, Albany, N Y *Senior Bacteriologist* (6, 1938)
- Cohn, Alfred E**, M D 300 Central Park W, New York City *Member, Rockefeller Institute for Medical Research* (1R, 1911, 3, 1913)
- Cohn, Clarence**, M D Michael Reese Hosp, Med Res Inst, Chicago, Ill *Director, Dept of Biochemistry* (1, 1948)
- Cohn, Edwin J**, Ph D, A M (Hon), Sc D (Hon) 183 Brattle St, Cambridge, Mass *Professor of Biological Chemistry, Harvard Medical School, Boston, Member, National Academy of Sciences* (1, 1919, 2, 1919)
- Cohn, Waldo E**, Ph D Oak Ridge National Lab, Oak Ridge, Tenn *Principal Biochemist* (2, 1944)
- Cole, Harold H**, M S, Ph D Division of Animal Husbandry, College of Agriculture, University of California, Davis *Professor* (1, 1947)
- Cole, Harold N**, Ph B, M D 1352 Hanna Bldg, Cleveland, O *Clinical Professor of Dermatology and Syphilology, Western Reserve University* (3, 1925)
- Cole, Kenneth S**, Ph D Institute of Radiobiology and Biophysics, University of Chicago, Chicago 37, Ill *Professor of Biophysics* (1, 1934)
- Cole, Rufus**, M D, D Sc Mount Kisco, N Y *Member Emeritus, Rockefeller Institute for Medical Research* (6, 1917)
- Cole, Versa V**, Ph D, M D Indiana University School of Medicine, 1040-1232 West Michigan St, Indianapolis *Associate Professor of Pharmacology* (3, 1911)
- Collett, Mary Elizabeth**, A M, Ph D Mather College, Western Reserve University, Cleveland, O *Associate Professor of Biology* (1, 1921)
- Collier, H Bruce**, M A, Ph D Dept of Biochemistry, Univ of Saskatchewan, Saskatoon, Sask *Professor of Biochemistry* (2, 1944)
- Collings, William Dojne**, Ph D Medical Laboratories, State University of Iowa, Iowa City (1, 1941)
- Collins, Dean A**, M A, Ph D, M D Temple Univ School of Medicine, 3400 N Broad St, Philadelphia 10, Pa *Associate Professor of Physiology* (1, 1938)
- Collins, Russell J**, A M, M D, F R C P (Can) M R C P (Edin) F A C P St John, New Brunswick, Canada *Medical Superintendent of St John Tuberculosis Hospital* (3, 1915)
- Collip, J B**, Ph D, D Sc, M D, C B E F R S C, I R S University of Western Ontario, London, Ontario, Canada *Dean, Medical Research* (1, 1920, 2, 1920)
- Colowick Sidney P**, Ph D Dept of Biological Chemistry, Univ of Illinois College of Medicine, Chicago *Associate Professor* (2, 1944)
- Coman, Dale R**, M D McManes Laboratory of Pathology, University of Pennsylvania School of Medicine, Philadelphia *Associate Professor of Pathology* (4, 1939)
- Comroe, Julius H, Jr**, M D University of Pennsylvania Graduate School of Medicine, Philadelphia *Professor of Physiology and Pharmacology* (1, 1943, 3, 1939)
- Conant, James B**, Ph D 5 University Hall, Cambridge, Mass *President, Harvard University, Member, National Academy of Sciences* (2, 1932)
- Concepcion, Isabelo**, M D 589 Zamora, Pasay, Rizal, Philippines *Faculty of Medicine, University Santo Tomas, Manila, Philippines Professor of Biochemistry and Nutrition* (1, 1919)
- Conklin, Ruth E**, M S, Ph D Vassar College, Poughkeepsie, N Y *Professor of Physiology* (1, 1940)
- Conn, Jerome W**, M D University of Michigan Medical School, Ann Arbor, Mich *Associate Professor of Internal Medicine* (5, 1942)
- Cook, Donald Hunter**, Ph D University of Miami, Coral Gables 34, Fla *Professor of Chemistry* (2, 1929)
- Cooke, Robert A**, A M, Sc D (hon), M D 60 E 58th St, New York City *Director, Department of Allergy, Roosevelt Hospital* (6, 1920)
- Coolidge, Thomas B**, M D, Ph D Abbot Hall, University of Chicago, Chicago 37, Ill *Asso-*

- ciate Professor, Dept of Biochemistry, and Walter G Zoller Memorial Dental Clinic (2, 1942)
- Coon, Julius M, Ph D Dept of Pharmacology, Univ of Chicago, Chicago 37, Ill Assoc Professor of Pharmacology, Director, Toxicity Lab (3, 1941)
- Coons, Callie Mae, Ph D Bureau of Human Nutrition and Home Economics, U S Dept of Agriculture, Washington, D C Assistant Chief (5, 1933)
- Cope, Otis M, M D University of Florida, School of Pharmacy, Gainesville, Fla (1, 1929)
- Copley, Alfred Lewin, M D Laboratory of Cellular Physiology, Dept of Biology, New York Univ, Washington Square, New York 3, N Y Research Associate (1, 1944)
- Corbin, Kendall B, M D 919 80th St, S W, Rochester, Minn Consultant in Neurology, Mayo Clinic, Professor of Neuroanatomy, Mayo Foundation (1, 1941)
- Corcoran, Arthur Curtis, C M, M D Cleveland Clinic Foundation, Cleveland 6, O (1, 1940)
- Corey, Edward Lyman, Ph D School of Medicine, University of Virginia, University, Va Assistant Professor of Physiology (1, 1931)
- Cori, Carl F, M D Washington University School of Medicine, Kingshighway and Euclid Ave, St Louis, Mo Professor of Pharmacology and Biochemistry, Member, National Academy of Sciences (2, 1925, 3, 1934)
- Cori, Gerty T, M D Washington University School of Medicine, St Louis, Mo Research Associate, Professor of Biochemistry (2, 1927, 3, 1934)
- Corley, Ralph Conner, Ph D Department of Chemistry, Purdue University, Lafayette, Ind Professor of Biochemistry (2, 1927)
- Corper, Harry J, M D, Ph D 1295 Clermont St, Denver, Colo Director of Research, National Jewish Hospital and Univ of Colorado School of Medicine (2, 1912)
- Corson, Samuel A, M S, Ph D Department of Physiology, Howard University School of Medicine, Washington, D C (1, 1943)
- Cotts, Gerhard K, M D Lynchburg State Colony, Colony, Virginia Clinical Director (3, 1937)
- Co Tui, Frank, M D New York University College of Medicine, 477 First Ave, New York City Associate Professor of Experimental Surgery (3, 1931)
- Cournand, André Frederic, M D Chest Service, Bellevue Hospital, CD Building, 1st Ave at 28th St, New York City Assistant Professor of Medicine, College of Physicians and Surgeons, Columbia University (1, 1944)
- Cowgill, George Raymond, Ph D, Sc D Yale University, New Haven, Conn Professor of Nutrition (1, 1923, 2, 1922, 5, 1933)
- Cox, Gerald J, Ph D University of Pittsburgh School of Dentistry, Pittsburgh, Pa Prof of Dental Res (2, 1930, 5, 1935)
- Cox, Herald R, Sc D Lederle Laboratories Division, Pearl River, N Y Director, Viral and Rickettsial Research (6, 1946)
- Cox, Warren M, Jr, Ph D Mead Johnson & Co, Evansville, Ind Director of Nutritional Research (2, 1935, 5, 1945)
- Craig, Francis Northrop, M A, Ph D Medical Division, Army Chemical Center, Md Physiologist (1, 1946)
- Craig, L C, Ph D Rockefeller Institute for Medical Research, 66th St and York Ave, New York City Associate Member (2, 1938)
- Crampton, E W, Ph D Macdonald College, McGill University, Quebec, Canada Professor of Nutrition (5, 1940)
- Crandall, Lathan A, Jr, M D, Ph D Miles Laboratories, Inc, Elkhart, Indiana (1, 1930, 5, 1940)
- Cranston, Elizabeth M, B A, M S, Ph D Dept of Pharmacology, Univ of Minnesota Medical School, Minneapolis 14, Minn Instructor, Dept of Pharmacology (3, 1946)
- Cravens, W W, M S, Ph D Poultry Department, University of Wisconsin, Madison Associate Professor of Poultry Husbandry (5, 1947)
- Craver, Bradford N, M A, Ph D, M D Ciba Pharmaceutical Products, Inc, Lafayette Park, Summit, New Jersey Senior Pharmacologist (3, 1946)
- Crescitelli, Frederick, Ph B, Sc M, Ph D Dept of Zoology, Univ of Calif, Los Angeles, Calif Physiologist (1, 1946)
- Cretcher, Leonard H, Ph D Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa Assistant Director and Head of the Department of Research in Pure Chemistry (2, 1930)
- Crider, Joseph O, M D Jefferson Medical College, Philadelphia, Pa Associate Professor of Physiology and Assistant Dean (1, 1935)
- Crisler, George R, Ph D, M D 157 E New England Ave, Winter Park, Fla (1, 1930)
- Crismon, Jefferson Martineau, M D Stanford University, Calif Assistant Professor of Physiology (1, 1944)
- Crittenden, Phoebe J, M S, Ph D Department of Physiology and Hygiene, Goucher College, Towson 4, Md (1, 1937, 3, 1937)
- Crozier, William J, Ph D Biological Laboratories, Harvard University, Cambridge, Mass Professor of General Physiology (1, 1928)
- Csonka, F A, Ph D Bureau of Human Nutrition and Home Economics, U S Department of Agriculture, Beltsville, Md Senior Chemist (2, 1924)

- Cullen, Stuart C, M D University Hospitals, Iowa City, Iowa *Assistant Professor of Surgery-Anesthesia* (3, 1944)
- Cunningham, Raymond W, M S, Ph D Lederle Laboratories, Inc, Pearl River, N Y *Head, Pharmacology Research* (3, 1941)
- Cunningham, Robert Sydney, A M, M D, Sc D Albany Medical College, Albany, N Y *Professor of Anatomy and Dean* (1, 1923)
- Cureton, Thomas Kirk Jr, M A, Ph D, B P E, M P E Univ of Illinois, School of Physical Education, Urbana, Ill *Associate Professor of Physical Education* (1, 1946)
- Curnen, Edward C, M D Yale Univ School of Medicine, New Haven 11, Conn (6, 1941)
- Curtis, George Morris, M A, Ph D, M D Kinsman Hall, Ohio State University, Columbus *Professor of Surgery, Chairman, Department of Research Surgery* (1, 1933, 4, 1933)
- Curtis, Howard J, M A, Ph D Vanderbilt University School of Medicine, Nashville 1, Tenn *Professor of Physiology* (1, 1940)
- Cutting, Reginald A, M D, Ph D Georgetown University School of Medicine, 3900 Reservoir Road, N W, Washington, D C *Professor of Physiology and Director of the Department* (1, 1939)
- Cutting, Windsor C, M D Stanford University School of Medicine, San Francisco, Calif *Assistant Professor of Therapeutics* (3, 1939)
- Daft, Floyd Shelton, Ph D National Institutes of Health, Bethesda, Md *Scientist Director, Chief, Nutrition Section, Assistant Chief, Division of Physiology* (5, 1941)
- Daggs, Ray Gilbert, Ph D Medical Dept, Field Research Lab, Fort Knox, Kentucky *Director of Research* (1, 1935, 5, 1933)
- Dakin, Henry D, D Sc, LL D, Ph D, F I C, F R S Scarborough-on-Hudson, N Y (2, 1906)
- Dalldorf, Gilbert, M D New York State Department of Health, Albany, N Y *Director, Division of Laboratories and Research* (4, 1947)
- Dalton, Albert J, M A, Ph D National Cancer Institute, Bethesda, Md *Principal Cytologist* (4, 1942)
- Dam, Henrik, D Sc Biologisk Afdeling Danmarks Tekniske Højskole, Østervoldgade 10 L, Copenhagen, K, Denmark *Professor* (2, 1944, 5, 1943)
- D'Amour, Fred E, M S, Ph D 2311 S Josephine St, Denver, Colo *Associate Professor, Department of Zoology, University of Denver* (1, 1934)
- D'Amour, Marie C, Ph D, M D 2311 So Josephine St, Denver, Colo (1, 1934)
- D'Angelo, Savino A, M S, Ph D Department of Biology, New York University, Washington Square, New York 3, N Y *Assistant Professor of Biology* (1, 1947)
- Daniels, Amy L, Ph D 720 N Van Buren St, Iowa City, Iowa *Retired* (2, 1919, 5, 1933)
- Danielson, Irvin S, Ph D Pearl River Apartments, Apt 3H, Pearl River, N Y *Research Chemist* (2, 1937)
- Dann, W J, Ph D, D Sc Duke University School of Medicine, Durham, N C *Professor of Nutrition* (2, 1937, 5, 1938)
- Danowski, T S, M D University of Pittsburgh School of Medicine, Pittsburgh, Pa *Renzhausen Professor of Research Medicine* (1, 1917)
- Darby, William J, M D, Ph D Vanderbilt Univ School of Medicine, Nashville, Tenn *Associate Professor of Biochemistry, Assistant Professor of Medicine* (5, 1915)
- Darling, Robert Croly, M D 157 Glenwood Ave, Leona, N Y Dept of Medicine, Columbia Univ College of Physicians and Surgeons, New York, 32, N Y (1, 1941)
- Darrow, Chester W, Ph D Institute for Juvenile Research, 907 S Wolcott St, Chicago, Ill *Research Psychologist, Institute for Juvenile Research, Associate in Physiology, University of Illinois College of Medicine* (1, 1937)
- Darrow, Daniel Cady, M D 789 Howard Ave, New Haven, Conn *Prof of Pediatrics, Yale Univ School of Medicine* (2, 1936)
- Daubert, B F, Ph D University of Pittsburgh, Pittsburgh, Penn *Research Professor and Research Administrator* (2, 1947)
- Davenport, Horace Willard, B S, B Sc (Oxon) Ph D Dept of Physiology, University of Utah, Salt Lake City 1 (1, 1942)
- David, Norman Austin, M D University of Oregon Medical School, Portland *Professor of Pharmacology* (3, 1934)
- Davidsohn, Israel, M D Mount Sinai Hospital, Chicago, Ill *Pathologist and Director of Laboratories, Chicago Medical School, Professor of Pathology and Chairman of Dept* (4, 1939, 6, 1929)
- Davies, Philip W, Ph D Univ of Pennsylvania, Johnson Res Found, Philadelphia, Pa *Assoc in Biophysics* (1, 1948)
- Davis, Bernard D, M D Cornell Univ Med College, New York City *Research Associate, USPHS, Surgeon* (6, 1948)
- Davis, George Kelso, Ph D Nutrition Laboratory, Animal Industry Dept, Agricultural Experiment Station, Gainesville, Fla *Nutritional Technologist and Biochemist, Professor of Nutrition, Univ of Florida, Florida Agricultural Experiment Station* (5, 1944)
- Davis, Hallowell, M D Central Institute for the Deaf, 818 S Kingshighway, St Louis 10, Mo (1, 1925)
- Davis, Harry A, M D, C M 2007 Wilshue Blvd, Los Angeles 5, Calif (4, 1944)



- Davis, John Emerson, M S , Ph D Univ of Arkansas School of Medicine, Little Rock Professor of Pharmacology and Physiology (1, 1941, 3, 1941)
- Dawson, Charles R , Ph D 246 Havemeyer Hall, Columbia University, New York City Assoc Prof of Chemistry (2, 1946)
- Dawson, James Robertson, Jr , M D Vanderbilt Medical School, Nashville, Tenn Professor of Pathology (4, 1940)
- Day, Harry G , D Sc Indiana University, Bloomington Associate Professor, Dept of Chemistry (5, 1940, 2, 1948)
- Day, Paul L , M A , Ph D University of Arkansas School of Medicine, Little Rock Professor of Physiological Chemistry (2, 1934, 5, 1933)
- De, N N , M B Indian Institute of Science, P O Malleswaram, Bangalore, India Asst Professor of Pharmacology (3, 1948)
- Dearborn, Earl H , M A , Ph D Johns Hopkins Univ School of Medicine, 800 N Washington St Baltimore 5, Md Instructor in Pharmacology and Experimental Therapeutics (3, 1946)
- de Beer, Edwin J , Ph D The Wellcome Research Laboratories, Tuckahoe, N Y Assistant Director of Research (3, 1944)
- De Bodo, Richard C , M D 477 First Ave , New York, N Y Associate Professor of Pharmacology, New York Univ College of Medicine (1, 1932, 3, 1931)
- De Boer, Benjamin, M A , Ph D St Louis University School of Medicine, 1402 South Grand Blvd , St Louis 4, Mo Assistant Professor of Pharmacology (1, 1947, 3, 1948)
- DeEds, Floyd, M A , Ph D 344 Santa Ana Ave , San Francisco, Calif Principal Pharmacologist, Western Regional Research Laboratory, 800 Buchanan St , Albany, Calif (2, 1937, 3, 1927)
- Defendorf, James Holmes, Ph D Office of the Chief of the Chemical Warfare Service, Washington, D C Colonel, Sn C (3, 1940)
- de Gara Paul F , M D 200 Pinehurst Ave , New York City Instructor in Pathology, Cornell University Medical College, Physician, New York Hospital (6, 1941)
- DeGraff, Arthur C , M D New York University College of Medicine, New York City Professor of Therapeutics (3, 1937)
- de Gutierrez-Mahoney, C G , M D St Vincent's Hospital, New York, N Y Director, Neurological Division and Neurosurgeon in Chief (1, 1940, 4, 1941)
- Deichmann, William B , M Sc , Ph D Albany Medical College, Albany, N Y Associate Professor of Pharmacology and Head, Division of Pharmacology (3, 1941)
- del Pozo, E C , M D Medellin 196, Mexico, D F , Mexico (1, 1943)
- Dempsey, Edward W , Sc M , Ph D Harvard Medical School, Boston, Mass Associate Professor of Anatomy (1, 1940)
- Denstedt, Orville F , Ph D McGill Univ , Montreal, Canada Asso Professor of Biochemistry (2, 1948)
- Derbyshire, Arthur J , Ph D EEG Department, Harper Hospital, Detroit, Mich (1, 1939)
- de Savitsch, Eugene, M D Suite 24, 1150 Connecticut Ave , Washington, D C Clinical Instructor in Surgery, Georgetown University School of Medicine Consulting Surgeon, Home of Incurables, Surgeon, Doctors Hospital (4, 1934)
- Dettwiler, Herman A , M S , Ph D Eli Lilly and Co , Indianapolis, Ind Assistant Director, Biological Division (6, 1946)
- Deuel, Harry J , Jr , Ph D University of Southern California Medical School, Los Angeles Professor of Biochemistry (1, 1928, 2, 1924, 5, 1933)
- Deulofeu, Venancio, D Chem Casilla Correo 2539, Buenos Aires, Argentina Professor of Organic Chemistry, University of Buenos Aires (2, 1942)
- Deutsch, Harold F , Ph D Univ of Wisconsin, Madison, Wis Asso Professor of Physiological Chemistry (2, 1948)
- Dey, Frederick L , Ph D , M D Box 11, Submarine Base, New London, Conn Lt (jg ), U S N R (1, 1945)
- Dickison, H L , M A , Ph D Bristol Laboratories, Inc , Building 6, Syracuse 1, N Y (3, 1946)
- Dieckmann, William J , M D The Chicago Lying In Hospital, 5841 Maryland Avenue, Chicago 37, Ill Mary Campau Ryerson Professor and Chairman of the Department of Obstetrics and Gynecology, University of Chicago (3, 1947)
- Dienes, Louis, M D Massachusetts General Hospital, Boston Bacteriologist (6, 1924)
- Dill, David Bruce, M A , Ph D Medical Division, Army Chemical Center, Md Scientific Director (1, 1941, 2, 1927, 5, 1936)
- Dille James M , M S , Ph D , M D Univ of Washington School of Medicine, Seattle 5, Wash Professor of Pharmacology, Assistant Dean (3, 1939)
- Dillon, Robert T , M S , Ph D % G D Searle and Co , Box 5110, Chicago 80, Ill Head, Analytical Division (2, 1934)
- Dingle John H , Sc D , M D Western Reserve University School of Medicine, Cleveland 6, Ohio Professor of Preventive Medicine (6, 1941)
- Di Palma, Joseph R , M D Long Island College of Medicine, 350 Henry St , Brooklyn, N Y Associate in Physiology (1, 1943)

- Dische, Zacharias, M D** Dept of Biochemistry, College of Physicians and Surgeons, 630 W 168th St, New York City (2, 1944)
- Dixon, Harold M, M D** Army Institute of Pathology, Washington 25, D C (1, 1936)
- Doan, Charles A, M D** Ohio State University, College of Medicine, Columbus Dean, Professor of Medicine, Director of Medical Research (4, 1928)
- Dobriner, Konrad, M D** Sloan-Kettering Institute, 444 E 68th St, New York 21, N Y Member (2, 1916)
- Dochez, A Raymond, M D, Sc D (hon)** Presbyterian Hospital, 620 W 168th St, New York City John E Borne Professor of Medical and Surgical Research, Columbia University, Member of National Academy of Sciences (1R, 1917, 6R, 1922)
- Dodds, Mary L, Ph D** Pennsylvania State College, State College, Pa Professor, Foods and Nutrition Research (5, 1918)
- Dohan, F Curtis, M D** 80 Princeton Rd, Cynwyd, Pa Fellow, George S Cox Medical Research Institute, Associate in Medicine, University of Pennsylvania, Philadelphia (1, 1911)
- Doisy, Edward A, M S, Ph D, Sc D** St Louis University School of Medicine, St Louis 4, Mo Professor of Biological Chemistry, Member, National Academy of Sciences (2, 1920)
- Dolman, C E, D P H, Ph D** The University of British Columbia, Vancouver, B C, Canada Head, Dept of Bacteriology and Preventive Medicine (6, 1947)
- Dominguez, Rafael, M D** Western Reserve University, Cleveland, Ohio Associate in Pathology, Director of Laboratories and Research, St Luke's Hospital (1, 1935)
- Donahue, D D, D Sc** Division of Industrial Hygiene, National Institute of Health, Bethesda, Md Physiologist, Toxicology Section, Division of Industrial Hygiene, U S Public Health Service (3, 1941)
- Donelson, Eva G, Ph D** The Ohio State University, Columbus, Ohio Professor of Home Economics (5, 1947)
- Dorfman, Albert, Ph D, M D** Univ of Chicago, Chicago, Ill Asst Professor of Pediatrics (2, 1948)
- Dorfman, Ralph I, Ph D** Dept of Biochemistry, Western Reserve University School of Medicine, Cleveland, O Assistant Professor of Biochemistry (2, 1940)
- Doti, Louis Basil, M A, Ph D** St Luke's Hospital, Amsterdam Ave and 113th St, New York City Chemist, St Luke's Hospital, Lecturer in Physiology and Biochemistry, New York Medical College (1, 1937)
- Doty, J Roy, Ph D** American Dental Association, 222 E Superior St, Chicago, Ill Senior Chemist (2, 1941)
- Doudoroff, Michael, Ph D** Dept of Bacteriology, 3531 Life Science Bldg, Univ of Calif, Berkeley, Calif Associate Professor of Bacteriology (2, 1916)
- Dounce, Alexander L, Ph D** Strong Memorial Hospital, 260 Crittenden Blvd, Rochester, N Y Instructor in Biochemistry, University of Rochester, School of Medicine and Dentistry (2, 1914)
- Dow, Philip, Ph D** University of Georgia School of Medicine, Augusta Associate Professor of Physiology (1, 1939)
- Dow, Robert S, M D, Ph D** University of Oregon Medical School, Portland Associate Professor of Anatomy (1, 1940)
- Downs, Ardrey W, M A, M D, D Sc, F A C P** University of Alberta, Edmonton, Alberta, Canada Professor of Physiology and Pharmacology (1, 1917)
- Downs, Cora M, Ph D** 1625 Alabama St, Lawrence, Kan (6, 1929)
- Dovle, William Lewis, M A, Ph D** 930 East 58th St, Chicago 37, Ill Associate Professor of Anatomy, University of Chicago (1, 1916)
- Drabkin, David L, M D** Graduate School of Medicine, University of Pennsylvania, Philadelphia Prof and Chr, Dept of Physiological Chemistry (2, 1928, 5, 1931)
- Dragstedt, Carl A, Ph D, M D** Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill Professor of Pharmacology (1, 1928, 3, 1932)
- Dragstedt, Lester R, M D, Ph D** University of Chicago, Chicago, Ill Professor of Surgery (1, 1920)
- Draize, J H, Ph D** Division of Pharmacology, Food & Drug Administration, Federal Security Agency, Washington, D C Pharmacologist (3, 1910)
- Drake, T G H, M B, F R C P (c)** University of Toronto, Toronto, Canada Junior Demonstrator in Pediatrics, Department of Medicine, University of Toronto, Clinical Assistant on Active Staff and Associate Director Research Laboratory, Hospital for Sick Children (5, 1936)
- Draper, William B, M Sc, M D** University of Colorado School of Medicine, 4200 E 9th Ave, Denver Associate Professor of Physiology and Pharmacology (1, 1947, 3, 1938)
- Dreisbach, Robert H, Ph D, M D** Stanford University School of Medicine, San Francisco 15, Calif Asst Professor (3, 1945)
- Dreyer, Nicholas Bernard, M A (Oxon)** School of Medicine, University of Vermont, Burlington Associate Professor of Physiology and Pharmacology (3, 1942)
- Drill, Victor Alexander, Ph D M D** Wayne University College of Medicine, Detroit 26, Mich Professor of Pharmacology (1, 1943, 3, 1946)

- Drinker, Cecil K**, M D Harvard University School of Public Health, Boston, Mass *Professor of Physiology and Dean* (1, 1915)
- Dripps, Robert D**, M D School of Medicine, University of Pennsylvania, Philadelphia 4 *1st Professor of Anesthesiology, Associate in Pharmacology* (1, 1947, 3, 1945)
- Driver, Robert L**, M S, Ph D University of Pennsylvania Hospital, Philadelphia, Pa (1, 1945, 3, 1947)
- Drury, Douglas R**, M D University of Southern California, Los Angeles *Professor of Physiology* (1, 1932)
- Dubin, Harry E**, Ph D 11 W 42nd St, New York 18, N Y *President, H E Dubin Laboratories, Inc* (2, 1925)
- Dubin, Isadore N**, M D Institute of Pathology, University of Tennessee, Memphis 7, Tenn *Associate Professor of Pathology and Bacteriology* (4, 1947)
- Dubnoff, Jacob W**, Ph D 1201 E California St, Pasadena 4, Calif *Senior Research Fellow, California Institute of Technology* (2, 1946)
- DuBois, Eugene F**, M D Cornell University Medical School, 1300 York Ave, New York, N Y *Professor and Head of the Department of Physiology and Biophysics, Attending Physician, New York Hospital, Member, National Academy of Sciences* (1, 1913, 3, 1921, 5, 1935)
- Du Bois, Kenneth P**, B S M S, Ph D Dept of Pharmacology, Univ of Chicago, Chicago 37, Ill *Asst Professor of Pharmacology* (3, 1946)
- Dubos, Rene J**, Ph D, D Sc Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Head, Dept of Bacteriology* (6, 1938)
- Dugal, L Paul**, M A, M Sc, Ph D Research Department on Acclimatization, Medical School, Laval University, Quebec City, Canada *Research Professor and Head of the Research Department on Acclimatization, Associate Director of the Institute of Hygiene* (1, 1947)
- Dukes, H H**, D V M, M S New York State Veterinary College, Cornell University, Ithaca, N Y *Professor of Veterinary Physiology* (1, 1934)
- Dulaney, Anna D**, A M, Ph D Pathological Institute, University of Tennessee, Memphis *Associate Professor of Bacteriology, Medical School* (6, 1924)
- Dumke, Paul Rudolph**, M D University of Pennsylvania Hospital, Philadelphia, Pa *Assistant Professor of Surgery* (3, 1942)
- Dunlap, Charles E**, M D Tulane University of Louisiana, 1430 Tulane Ave New Orleans *Professor of Pathology and Bacteriology* (4, 1942)
- Dunn, Max Shaw**, Ph D University of California, Los Angeles *Professor of Chemistry* (2, 1933)
- Dunn, Thelma Brumfield**, M D The National Cancer Institute, Bethesda, Md *Pathologist* (4, 1945)
- Dury, Abraham**, Ph D George Washington Univ School of Med, Washington, D C *Asst Professor of Physiology* (1, 1948)
- Dutcher, James D**, M S, Ph D The Squibb Institute for Medical Research, New Brunswick, New Jersey *Research Associate, Division of Organic Chemistry* (2, 1946)
- Dutcher, R Adams**, M S, M A, D Sc Pennsylvania State College, State College *Professor and Head of Department of Agricultural and Biological Chemistry* (2, 1920, 5, 1933)
- Duval, Charles Warren**, M D Pensacola Hospital, Pensacola, Fla (4, 1913)
- du Vigneaud, Vincent**, M S, Ph D Cornell University Medical College, 1300 York Ave, New York 21, N Y *Professor of Biochemistry, Member, National Academy of Sciences* (2, 1929, 5, 1934)
- Dworkin, Simon**, D D S, M D, C M Biology Building, McGill University, Montreal, Quebec, Canada *Lecturer in Physiology, Faculty of Medicine* (1, 1931)
- Dye, J A**, Ph D James Law Hall, Cornell University, Ithaca, N Y *Professor of Physiology* (1, 1929)
- Dye, Marie**, M S, Ph D Michigan State College, East Lansing *Dean, School of Home Economics* (2, 1929, 5, 1933)
- Dyer, Helen M**, M S, Ph D National Cancer Institute, N I H, U S P H S, Bethesda, Md *Biochemist* (2, 1936, 5, 1937)
- Dziemian, Arthur J**, Ph D Army Chemical Center, Medical Division, Edgewood, Md *Physiologist, Biophysics Section* (1, 1948)
- Eadie, George S**, Ph D Duke University School of Medicine, Box 3709, Durham, N C *Professor of Physiology and Pharmacology* (1, 1929, 3, 1940)
- Eagle, Harry**, A B, M D United States Public Health Service, Bethesda, Md *Medical Director, Scientific Director, National Cancer Institute* (3, 1946, 4, 1936)
- Eakin, Robert E**, Ph D Dept of Chemistry, Univ of Texas, Austin, Texas *Asso Professor of Chemistry* (2, 1948)
- Earle, D P, Jr**, M D, Med Sc D New York University College of Medicine, 477 First Avenue, New York 16, N Y *Associate Professor of Medicine* (1, 1947)
- Earle, Wilton R**, Ph D U S Public Health Service, National Cancer Institute, Bethesda, Md *Principal Cytologist* (4, 1940)
- Eaton, Monroe D**, M D Harvard Medical School Boston, Mass *Assoc Professor of Bacteriology and Immunology* (6, 1937)
- Eckenhoff, James E**, M D Univ of Pennsylvania School of Medicine, Philadelphia, Pa

- Asso in Clinical Pharmacology and Anesthesiology* (1, 1948)
- Ecker, E E**, Ph D School of Medicine, Western Reserve University, 2085 Adelbert Rd, Cleveland, O *Professor of Immunology* (1, 1925, 6, 1947)
- Eckstein, Gustav**, M D Univ of Cincinnati College of Med, Cincinnati, Ohio *Asso Professor of Physiology* (1, 1948)
- Eckstein, Henry C**, M S, Ph D 320 W Medical Building, University of Michigan, Ann Arbor *Associate Professor of Biological Chemistry* (2, 1925)
- Eckstein, R W**, M A, M D Department of Medicine, Western Reserve University, Cleveland, Ohio *Senior Instructor, in charge of Cardiovascular Experimental Medical Research Laboratory* (1, 1947)
- Eddy, Nathan B**, M D National Institute of Health, Bethesda, Md *Principal Pharmacologist, United States Public Health Service* (3, 1929)
- Eddy, Walter H**, A M, Ph D American Chlorophyll, Inc, Lake Worth, Fla *Scientific Director* (2, 1913, 5, 1933)
- Edelmann, Abraham**, Ph D Brookhaven National Laboratories, Upton, L I, N Y *Physiologist* (1, 1948)
- Edholm, O G**, M B Univ of Western Ontario Med School, London, Canada *Head, Dept of Physiology* (1, 1948)
- Edsall, Geoffrey**, M D Antitoxin and Vaccine Laboratory, 375 South St, Jamaica Plain, Mass *Acting Director, Division of Biologic Laboratories, Massachusetts Department of Public Health, Associate in Public Health Laboratory Methods, Simmons College, Instructor in Applied Immunology, Harvard School of Public Health* (6, 1943)
- Edsall, John Tileston**, M D Harvard Medical School, Boston, Mass *Associate Professor of Biological Chemistry and Tutor in Biochemical Sciences* (2, 1931)
- Edwards, Dayton J**, Ph D Cornell University Medical College, 1300 York Ave, New York City *Associate Professor of Physiology, Assistant Dean* (1, 1921)
- Edwards, Jesse E**, M D Mayo Clinic, (Sect Path Anat), Rochester 4, Minn *Assistant Professor of Pathologic Anatomy, Mayo Foundation, Graduate School, University of Minnesota* (4, 1941)
- Edwards, J Graham**, A M, Ph D 24 High St, Buffalo, N Y *Assistant Professor of Anatomy, University of Buffalo* (1, 1932)
- Eggerth, Arnold H**, Ph D Hoagland Laboratory, 335 Henry St, Brooklyn, N Y *Associate Professor of Bacteriology, Long Island College of Medicine* (4, 1925)
- Ehrenstein, Maximilian R**, Ph D 806 Maloney Clinic, University of Pennsylvania Hospital, Philadelphia, Pa *Assoc Prof of Physiological Chemistry assigned to Medicine, Univ of Pennsylvania School of Medicine* (2, 1912)
- Ehrich, William E**, M D University of Pennsylvania Medical School, Philadelphia *Professor of Histology and Pathology, Graduate School of Medicine, Chief, Division of Pathology, Philadelphia General Hospital* (1, 1945)
- Eichelberger, Lillian**, Ph D University of Chicago, Dept of Medicine, Chicago, Ill *Associate Professor of Biochemistry* (2, 1937)
- Eiler, John J**, Ph D Univ of California Medical Center, San Francisco, Calif *Assoc Professor of Biochemistry and Pharmacology, Univ of California Coll of Pharmacy* (2, 1948)
- Eisenman, Anna J**, Ph D U S Public Health Service Hospital, Lexington, Ky *Biological Chemist* (2, 1930)
- Elberg, Sanford S**, Ph D Univ of California, Berkeley, Calif *Assoc Professor of Bacteriology* (6, 1918)
- Elderfield, Robert C**, Ph D Columbia University, New York City *Professor of Chemistry* (2, 1934)
- Elftman, Herbert**, M A, Ph D College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York City *Assistant Professor in Anatomy* (1, 1940)
- Eliot, Martha M**, M D United States Children's Bureau, Washington 25, D C *Associate Chief* (5, 1933)
- Elkinton, J Russell**, M D University of Pennsylvania Hospital, Philadelphia, Pa *Dept of Medicine* (1, 1917)
- Elliott, Henry W**, Ph D Univ of California Medical School, San Francisco, Calif *Instructor in Pharmacology and Lecturer in Dental Pharmacology and Toxicology* (3, 1948)
- Elliott, K Allan C**, M Sc, Ph D Montreal Neurological Institute, 3801 University St, Montreal, Canada *Assistant Professor in Neurology, Biochemistry, McGill University* (2, 1937)
- Ellis, C H**, M S, Ph D Laboratories of Gordon A Alles, 770 S Arroyo Parkway, Pasadena 5, Calif *Research Associate in Physiology* (1, 1947)
- Ellis, Fred W**, M S, Ph D University of North Carolina, Chapel Hill *Assistant Professor of Pharmacology* (3, 1945)
- Ellis, Lillian N**, Ph D Adelphi College, Garden City, N Y *Instructor in Chemistry* (5, 1940)
- Ellis, Max Mapes**, A M, Ph D, Sc D Medical School, University of Missouri, Columbia *Professor of Physiology and Pharmacology* (1, 1923)
- Ellis, N R**, M S Bureau of Animal Industry,

- U S Department of Agriculture, Agricultural Research Center, Beltsville, Md *Asst Chief, Animal Husbandry Division* (2, 1928, 5, 1933)
- Ellis, Sydney, M A, Ph D Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N C *Assistant Professor of Pharmacology* (3, 1947)
- Elrod, Ralph P, M S, Ph D University of South Dakota Medical School, Vermillion, S D *Chairman of Department of Microbiology* (6, 1947)
- Elser, William J, M D Kent, Conn (6, 1920)
- Elvehjem, Conrad Arnold, M S, Ph D, Sc D Biochemistry Building, University of Wisconsin, Madison *Chairman, Dept of Biochemistry, Dean of Graduate School, Member, National Academy of Sciences* (2, 1931, 5, 1933)
- Embree, Norris Dean, Ph D Distillation Products, Inc, 755 Ridge Road West, Rochester 13, N Y *Acting Director of Research* (2, 1946)
- Emerson, George A, M S, Ph D University of Texas, Medical Branch, Galveston *Professor of Pharmacology* (3, 1935)
- Emerson, Gladys A, Ph D Merck Institute of Therapeutic Research, Rahway, N J *Nutritionist* (5, 1942)
- Emerson, Oliver H, Ph D Western Regional Research Laboratory, U S Dept of Agriculture, Albany 6, Calif *Associate Chemist* (2, 1938)
- Emery, Frederick E, D V M, M S, Ph D University of Arkansas School of Medicine, Little Rock *Professor of Physiology and Pharmacology* (1, 1930)
- Emmart, Emily W, M A, Ph D National Institute of Health, Bethesda 14, Md (3, 1947)
- Emmett, Arthur D, M A, Ph D Star Route 1, Lewiston, Mich (2, 1908, 5, 1933)
- Enders, John F, A M, Ph D Department of Bacteriology, Medical School, Harvard University, Boston, Mass *Associate Professor of Bacteriology and Immunology, Chief, Research Division of Infectious Diseases Children's Hospital* (6, 1936)
- Endicott, Kenneth M, M D National Institute of Health, Bethesda 14, Md *Surgeon, U S Public Health Service, Division of Pathology* (4, 1947)
- Engel, Frank L, M D Duke University School of Medicine, Durham, North Carolina *Associate in Medicine* (1, 1947)
- Engel, Ruben W, Ph B, Ph D Lab of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala *Animal Nutritionist* (5, 1948)
- Engle, Earl Theron Ph D College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York City *Professor of Anatomy* (1, 1930)
- English, James, Jr, Ph D Sterling Chemistry Laboratory, Yale Univ, New Haven, Conn *Assistant Professor of Chemistry* (2, 1946)
- Enright, John B, Ph D State of California Dept of Public Health, Berkeley, Calif *Supervising Bacteriologist* (6, 1948)
- Epstein, Albert A, M D 1111 Madison Ave, New York City *Physician, Beth Israel Hospital, Physician, Hospital for Joint Diseases* (2, 1912)
- Ercoli, N, M D, Ph D Warner Institute for Therapeutic Research, New York 11, N Y *Director of Pharmacological Research* (3, 1947)
- Erickson, Cyrus C, M D Duke University School of Medicine, Durham, N C *Associate Professor of Pathology* (4, 1941)
- Erickson, John Otto, Ph D Atomic Energy Commission Project, Univ of Rochester School of Medicine, Rochester, N Y *Research Biochemist* (6, 1946)
- Eschenbrenner, Allen B, M D *Surgeon USPHS, National Institute of Health, Bethesda 14, Md* (4, 1946)
- Espe, Dwight L, Ph D Department of Dairy Husbandry, State College Station, Fargo, N Dak (1, 1940)
- Essex, Hiram E, M S, Ph D Mayo Foundation, Rochester, Minn *Professor of Physiology Institute of Experimental Medicine* (1, 1932, 3, 1940)
- Eitinger, C H, M D, C M, F R S C Queen's University, Kingston, Canada *Professor of Physiology, Assistant Director, Division of Medical Research, National Research Council, Ottawa* (1, 1943)
- Evans, Earl Alison, Jr, Ph D Department of Biochemistry, University of Chicago, Chicago, Ill *Professor and Chairman of Department* (2, 1939)
- Evans, Everett Idris, M D, Ph D Department of Surgery, Medical College of Virginia, Richmond *Assistant Professor of Surgery* (1, 1935)
- Evans, Gerald Taylor, M D, Ph D University of Minnesota Hospitals, Minneapolis *Director of Laboratory Service, University of Minnesota Hospitals, Associate Professor of Medicine, University of Minnesota* (1, 1942)
- Evans, Herbert M, M D University of California, Berkeley *Professor of Anatomy and Director of Institute of Experimental Biology, Member of the National Academy of Sciences* (1, 1919)
- Eveleth, D F, Ph D, D V M North Dakota Agricultural College, Fargo *Professor, Veterinary Science, North Dakota Agricultural Experiment Station* (2, 1939)
- Everett, Mark R Ph D University of Oklahoma School of Medicine, Oklahoma City

- Dean and Professor of Biochemistry (2, 1929)
- Everson, Gladys**, Ph D Iowa State College, Ames, Iowa Assoc Professor of Nutrition (5, 1948)
- Ewing, P L**, M S, Ph D University of Texas School of Medicine, Galveston Associate Professor of Pharmacology (3, 1938)
- Eyster, John A English**, M D University of Wisconsin, Madison Professor of Physiology (1, 1906, 3R, 1908)
- Fahr, George**, M D 102 Millard Hall, Univ of Minnesota, Minneapolis Professor of Clinical Medicine (1, 1913, 3, 1940)
- Failey, Crawford F**, Ph D 5454 South Shore Drive, Chicago 15, Ill Professor of Biochemistry, University of Chicago (2, 1933)
- Fairhall, Lawrence T**, M A, Ph D U S Public Health Service, Washington, D C Principal Industrial Toxicologist (2, 1924)
- Falk, Carolyn R**, B A 40 E 66th St, New York City Bacteriologist, Bureau of Laboratories, New York City Dept of Health (6, 1943)
- Falk, K George**, Ph D 40 E 66th St, New York City Director, Laboratory of Industrial Hygiene (2, 1913)
- Farah, Alfred E**, M D Univ of Washington School of Medicine, Seattle, Wash Asst Professor of Pharmacology (3, 1948)
- Farber, Sidney**, M D Harvard Medical School, 25 Shattuck St, Boston, Mass Assistant Professor of Pathology (4, 1934)
- Farmer, Chester J**, A M Northwestern Medical School, 303 E Chicago Ave, Chicago, Ill Professor of Chemistry (2, 1935)
- Farr, Lee E**, M D Alfred I duPont Institute, Wilmington, Del Director of Research, Pediatrician-in-Chief (4, 1941)
- Farrar, George E, Jr**, M D Chief, Medical Service A, Episcopal Hospital, 3401 N Broad Street, Philadelphia 40, Pennsylvania Associate Professor of Medicine, Temple University and Hospital (3, 1947)
- Farrell, James L**, Ph D, M D 636 Church St, Evanston, Illinois (1, 1938)
- Fassett, David W**, A B, M D Eastman Kodak Co, Lab of Industrial Medicine, Kodak Park Works, Rochester, N Y (3, 1942)
- Fay, Marion**, M A, Ph D Woman's Medical College of Pennsylvania, Philadelphia 29 Professor of Physiological Chemistry and Dean (2, 1937)
- Featherstone, Robert M**, M S, Ph D College of Medicine, State University of Iowa, Iowa City, Iowa Assistant Professor of Pharmacology (3, 1947)
- Feigen, George A**, A B Dept of Pharmacology and Toxicology, University of Southern California, School of Medicine, Los Angeles 7 (1, 1948)
- Feldman, Harry A**, M D Children's Hospital Research Foundation, Cincinnati, Ohio Fellow, National Research Council (6, 1913)
- Feldman, William H**, D V M, M S The Mayo Foundation, Rochester, Minn Professor in the Division of Experimental Surgery and Pathology (4, 1931)
- Fell, Norbert**, Ph D Camp Detrick, Frederick, Maryland Chief, Pilot Plant Division (6, 1911)
- Feller, A E**, M D Western Reserve School of Medicine, Cleveland 6, Ohio Associate Professor of Preventive Medicine (6, 1913)
- Fellows, Edwin J**, M S, Ph D Temple University School of Medicine, Philadelphia, Pa Assistant Professor of Pharmacology (3, 1939)
- Felton, Lloyd D**, M D, D Sc National Institute of Health, Bethesda, Md Medical Director, USPHS (6, 1926)
- Fenn, Wallace Osgood**, A M, Ph D University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y Professor of Physiology, Member, National Academy of Sciences (1, 1924)
- Fenning, Con**, M D, M A University of Utah School of Medicine, Salt Lake City Professor of Pharmacology and Physiology (1, 1942)
- Fenton, P F**, M S, Ph D Department of Physiological Chemistry, Yale University School of Medicine, New Haven 11, Conn Research Assistant (1, 1917)
- Ferguson, James Kenneth Wallace**, M A, M D 76 Kilbarry Rd, Toronto, Ontario, Canada Professor of Pharmacology, University of Toronto, Wing Commander, R C A F (1, 1933, 3, 1941)
- Ferguson, John Howard**, M D, M A, L M S S A Dept of Physiology, School of Medicine, University of North Carolina, Chapel Hill Professor of Physiology and Acting Professor of Pharmacology (1, 1933)
- Ferguson, L Kraeer**, M D 133 S 36th St, Philadelphia 4, Pa Professor of Surgery, Graduate School, Univ of Pennsylvania, and Woman's Medical College of Pennsylvania, Surgeon, Doctors Hospital and Woman's Medical College, Graduate Hosp, Philadelphia General Hospital (4, 1935)
- Ferry, John Douglass**, Ph D Univ of Wisconsin, Madison, Wis Professor of Chemistry (2, 1941)
- Ferry, Ronald M**, M D 966 Memorial Drive, Cambridge, Mass Associate Professor of Biochemistry, Harvard University (2, 1924)
- Fetcher, Edwin S**, Ph D R D #4, Xenia, Ohio (1, 1944)
- Fetter, Dorothy**, Ph D Department of Hygiene, Brooklyn College, Brooklyn, N Y Instructor in Physiology (1, 1944)
- Fevold, Harry L**, M S, Ph D 1849 W Pershing Road, Chicago 9, Ill Chief, Food Research

- Division, Quartermaster Food and Container Institute* (2, 1942)
- Field, John, II, A M**, Ph D Department of Physiology, Stanford University, Stanford, Calif *Professor of Physiology* (1, 1930)
- Fincke, Margaret L**, Ph D Oregon State College, Corvallis *Associate Professor of Foods and Nutrition, School of Home Economics* (5, 1940)
- Findley, Thomas, Jr**, M D Ochsner Clinic, 3503 Prytania, New Orleans, La *Head of the Department of Internal Medicine, Ochsner Clinic, New Orleans, Assistant Professor of Clinical Medicine, Tulane University School of Medicine* (1, 1938)
- Finerty, John C**, M S, Ph D Department of Anatomy, Washington University School of Medicine, St Louis 10, Mo *Assistant Professor of Anatomy* (1, 1947)
- Finland, Maxwell**, M D Boston City Hospital, Boston, Mass *Assistant Professor of Medicine, Harvard Medical School* (6, 1941)
- Finnegan, J K**, Ph D Medical College of Virginia, Richmond 19, Va *Assistant Professor of Pharmacology* (3, 1947)
- Flor, Warfield Monroe**, M D Johns Hopkins Hospital, Baltimore, Md *Associate Professor of Surgery, Johns Hopkins University* (1, 1932)
- Fischel, Edward E**, M D, D Med Sc Columbia Univ, New York City *Fellow in Medicine* (6, 1948)
- Fischer, Ernst**, M D, Dr habil Medical College of Virginia, Richmond *Professor of Physiology* (1, 1936)
- Fischer, Hermann O L**, Ph D Banting Institute, 100 College St, University of Toronto, Toronto 5, Canada *Research Professor of Organic Chemistry* (2, 1940)
- Fischer, Martin H**, M D, Pharm D (hon), Sc D University of Cincinnati College of Medicine, Eden Ave, Cincinnati 19, O *Professor of Physiology* (1, 1901, 2, 1919)
- Fishberg, Ella H**, M A, M D Beth Israel Hospital, Stuyvesant Park East, New York City *Biochemist* (2, 1931)
- Fisher, Albert Madden**, Ph D Connaught Med Res Laboratories, University of Toronto, Toronto, Canada *Research and Administrative Associate* (2, 1944)
- Fisher, Kenneth C**, M A, Ph D University of Toronto, Toronto, Ont, Canada *Assistant Professor of Physiological Zoology* (1, 1940)
- Fishman, William H**, Ph D Tufts College Med Sch, 30 Bennet St, Boston, Mass *Res Prof of Biochemistry* (2, 1947)
- Fiske, Cyrus H**, M D Harvard Medical School, Boston, Mass *Professor of Biological Chemistry* (2, 1914)
- Fitzhugh, O Garth**, Ph D Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D C *Pharmacologist* (3, 1940)
- Fleischmann, Walter**, M D, Ph D Medical Division, Army Chemical Center, Md *Chief, Physiology Section, Instructor in Pediatrics, Johns Hopkins University* (1, 1940)
- Fleisher, Moyer S**, M D Jewish Hospital, St Louis, Mo *Research Bacteriologist* (4, 1924, 6, 1932)
- Flexner, Louis B**, M D Carnegie Labs, Carnegie Institution of Washington, Wolfe and Madison Sts, Baltimore, Md *Research Associate, Department of Embryology* (1, 1933, 2, 1948)
- Flock, Eunice V**, Ph D Mayo Clinic, Rochester, Minn *Associate Professor in Experimental Medicine, Mayo Foundation, Univ of Minnesota* (2, 1940)
- Florman, Alfred L**, M D Mt Sinai Hospital, New York City *Investigator in Virology* (6, 1942)
- Flosdorf, Earl W**, Ph D Forest Grove, Pa University of Pennsylvania School of Medicine *Director of Research* (6, 1941)
- Floyd, Cleveland**, M D, Sc D 246 Marlborough St, Boston, Mass *Chief Examiner, Boston Health Dept* (6, 1916)
- Foa, Piero Pio**, Ph D 710 S Wolcott St, Chicago Ill *Associate Professor of Physiology and Pharmacology, Chicago Medical School* (1, 1944)
- Folch, Jordi**, M D McLean Hospital, Waverly, Mass *Assistant Professor of Biological Chemistry, Harvard Medical School Director of Scientific Research, McLean Hospital* (2, 1941)
- Folkers, Karl**, Ph D Merck and Co, Inc Rahway, N J *Director of Organic and Biochemical Research* (2, 1947)
- Follensby, Edna M**, Ph G 80 E Concord St, Boston, Mass *Research Assistant, Evans Memorial Special Instructor in Biology, Simmons College* (6, 1933)
- Folles, Richard H**, Jr, M D School of Medicine, Johns Hopkins University *Associate Professor of Pathology* (4, 1942)
- Fontaine, Thomas Davis**, Ph D Agricultural Research Center, Beltsville, Md *Biochemist and Acting Head, Div of Biologically Active Compounds* (2, 1946)
- Foot, Nathan Chandler**, M D Cornell Univ Med Coll, New York City *Emeritus Professor, Surgical Pathology* (4, 1924)
- Forbes, Alexander, A M**, M D Harvard Medical School, Boston, Mass *Professor of Physiology, Member of the National Academy of Sciences* (1, 1910)
- Forbes, Ernest B**, Ph D State College, Pa *Professor Emeritus Animal Nutrition* (1R, 1917, 5, 1935)

- Forbes, John C**, M A, Ph D Medical College of Virginia, Richmond *Research Professor of Biochemistry* (2, 1937)
- Forbes, William H**, M A, Ph D Harvard University, Fatigue Laboratory, Boston, Mass *Research Fellow, Assistant Director of Fatigue Lab, Assistant Professor of Industrial Physiology* (1, 1943)
- Forster, Francis M**, M D Jefferson Medical College, Philadelphia, Pa *Asst Professor of Neurology* (1, 1948)
- Fosdick, Leonard S**, Ph D 311 E Chicago Ave., Chicago, Ill *Professor of Chemistry, Northwestern University* (2, 1944)
- Foster, G L**, Ph D College of Physicians and Surgeons, 630 W 168th St., New York City *Professor of Biological Chemistry* (2, 1923)
- Foster, Harry E**, M D Fairmont Hotel, San Francisco, Calif (6, 1913)
- Foster, Jackson W**, Ph D Dept of Botany and Bacteriology, Univ of Texas, Austin 12, Texas *Associate Professor of Bacteriology* (2, 1916)
- Foster, Robert H K**, Ph D, M D St Louis University School of Medicine, St Louis, Mo *Professor and Director of Dept of Pharmacology* (1, 1940, 3, 1944)
- Fothergill LeRoy D**, M D Camp Detrick, Frederick, Maryland *Asst Technical Director* (6, 1936)
- Fox, Sidney W**, Ph D Chemistry Dept., Iowa State College, Ames, Iowa *Professor of Chemistry, Research Professor, Chemistry Section, Iowa Agricultural Experiment Station, Research Professor of Chemistry Industrial Science Research Institute, Iowa State College* (2, 1916)
- Fraenkel-Conrat, Heinz**, M D, Ph D Western Regional Research Laboratory, U S Dept of Agriculture, Albany 6, Calif *Chemist* (2, 1942)
- Francis, Thomas, Jr**, M D, M S (hon.), Sc D (hon.) School of Public Health, University of Michigan, Ann Arbor *Henry Sewall Prof of Epidemiology* (4, 1940, 6, 1930)
- Franke, Florent E**, M D 9 Sylvester, Webster Groves, Mo *Assistant Professor of Physiology, St Louis University School of Medicine* (1, 1934)
- Frankel, Edward M**, Ph D 214 River Rd., Nyack, N Y *Consulting Chemist* (2, 1916)
- Fraps, R M**, Ph D Bureau of Animal Husbandry, Beltsville, Md *Senior Physiologist* (1, 1947)
- Fraser, Alexander MacLeod**, A M, M D, C M Dalhousie University, Halifax, Nova Scotia *Professor of Pharmacology* (3, 1939)
- Fraser, Donald T**, M B, D P H, F R S C Connaught Laboratories, University of Toronto, Toronto 5, Canada *Professor of Hygiene and Preventive Medicine* (6, 1935)
- Frear, Donald E H**, M S, Ph D Dept of Agricultural and Biological Chemistry The Pennsylvania State College, State College, Pa *Professor of Agriculture and Biological Chemistry* (2, 1916)
- Fredette, Victor** Univ of Montreal, Montreal, Canada *Asst Professor of Bacteriology, Asso Director, Inst of Microbiology* (6, 1948)
- Free, Alfred H**, M S, Ph D Research Laboratory, Miles Laboratories, Inc., Elkhart, Ind *Head of Biochemistry Section* (2, 1916, 5, 1944)
- Freeman, Harry**, M D Worcester State Hospital, Worcester, Mass *Internist, Research Service* (1, 1939)
- Freeman, Leslie Willard**, Ph D, M D University of Indiana, Dept of Surgery, Indianapolis, Ind *Asst Professor of Surgery, Director of Surgical Research* (1, 1941)
- Freeman, Norman E**, M D Department of Surgery University of California Medical School, San Francisco 22 (1, 1936)
- Freeman, Smith**, M D, Ph D Northwestern University School of Medicine, 303 E Chicago Ave., Chicago, Ill *Assistant Professor of Physiology and Pharmacology* (1, 1937)
- French, Cyrus E**, Ph D Pennsylvania State College, State College, Pa *Asso Professor of Animal Nutrition* (5, 1948)
- French, C Stacy**, Ph D Carnegie Institution of Washington, Stanford University, Calif *Director, Division of Plant Biology* (1, 1947, 2, 1916)
- Freund, Jules**, M D Public Health Research Institute of the City of New York, New York City *Member and Chief of the Division of Applied Immunology* (1, 1930, 6, 1921)
- Frey, Charles N**, Ph D, Dr S 45 Cambridge Rd., Scarsdale, N Y *Director, Scientific Relations, Standard Brands Inc* (5, 1948)
- Fried, Josef**, Ph D Squibb Institute for Medical Research, New Brunswick, N J *Asso Member, Division of Organic Chemistry* (2, 1948)
- Friedman, Townsend B** Children's Memorial Hospital, Chicago, Ill *Attending Allergist* (6, 1918)
- Friedemann, Theodore E**, M A, Ph D Northwestern University Medical School, 303 E Chicago Ave., Chicago, Ill *Associate Professor of Physiology* (2, 1925)
- Friedemann, Ulrich**, M D Department of Bacteriology, The Jewish Hospital of Brooklyn, Classon and St Marks Ave., Brooklyn, N Y (6, 1938)
- Friedenwald, Jonas S**, M D, M A 1212 Eutaw Place, Baltimore 17, Md The Johns Hopkins Hospital Baltimore *Associate Professor of Ophthalmology* (1, 1947)
- Friedewald, William F**, M D Emory University School of Medicine, Atlanta, Ga *Professor of*



- Bacteriologist, Associate Professor of Medicine* (4, 1941)
- Friedgood, Harry B, M D University of California, Los Angeles, Calif *Assoc Clin Professor of Medicine, California Inst of Cancer Research, President and Chairman, Cedars of Lebanon Hospital, Consultant, Inpatient Staff Dept of Medicine* (1, 1936)
- Friedman, Maurice H, Ph D, M D 2040 Belmont Rd, Washington 9, D C (1, 1929)
- Friedman, Meyer, M D Harold Brunn Institute for Cardiovascular Research, Mt Zion Hospital, 2200 Post St, San Francisco, Calif *Director* (1, 1947)
- Friedman, M H F, M A, Ph D Jefferson Medical College of Philadelphia, 1025 Walnut St, Philadelphia, Pa *Assoc Professor of Physiology* (1, 1941)
- Friedman, Nathan B, M D Cedars of Lebanon Hospital, Los Angeles, Calif (4, 1942)
- Friedman, Sydney M, M D, Ph D Department of Anatomy, McGill University, Montreal 2, Canada *Assistant Professor of Anatomy* (1, 1947)
- Frost, Douglas Van Anden, Ph D Abbott Laboratories, North Chicago, Ill *Head of Nutritional Research* (2, 1946, 5, 1947)
- Fruton, J S, Ph D Yale School of Medicine, 333 Cedar St, New Haven, Conn *Associate Professor of Physiological Chemistry* (2, 1938)
- Fugo, Nicholas W, M S, Ph D Department of Obstetrics and Gynecology, University of Chicago, Chicago, Ill (3, 1944)
- Fuhrman, Frederick A, M S, Ph D Dept of Physiology, Stanford Univ, Stanford University, Calif *Instructor in Physiology* (1, 1946)
- Fulton, John Farquhar, M A, Ph D, M D Yale University School of Medicine, New Haven, Conn *Sterling Professor of Physiology* (1, 1925)
- Funk, Casimir, D Sc, Ph D 186 Riverside Drive, New York 24, N Y (2, 1921)
- Furchgott, Robert F, Ph D Cornell Univ Medical College, New York City *Asst Professor of Biochemistry in Medicine* (2, 1948)
- Furth, Jacob, M D Veterans Administration Hospital, Dallas, Texas *Chief of Laboratory* (4, 1932, 6, 1930)
- Gaebler, Oliver H, Ph D, M D Henry Ford Hospital, Detroit, Mich *Head, Department of Biochemistry, Edsel B Ford Inst for Med Res* (2, 1927)
- Gaffron, Hans, Ph D Department of Chemistry, University of Chicago, Chicago, Ill *Associate Professor of Biochemistry* (2, 1941)
- Gagge, Adolf Pharo, Ph D Aeromedical Research Laboratory, Wright Field, Dayton, O *Lt Col, Chief, Biophysics Branch, Air Corps, U S Army, on leave from Yale University and John B Pierce Laboratory of Hygiene* (1, 1939)
- Galambos, Robert, Ph D, M D Psycho-Acoustic Laboratory, Research Fellow, Harvard University (1, 1942)
- Gall, Edward A, M D Cincinnati General Hospital, Cincinnati, O *Assistant Professor of Pathology, College of Medicine, University of Cincinnati* (4, 1941)
- Gallagher, Thomas F, Ph D Sloan-Kettering Institute, 444 E 68th St, New York 21, N Y *Member* (2, 1932)
- Gallup, Willis D, M S, Ph D Oklahoma Agricultural and Mechanical College, Stillwater *Chemist and Professor of Agricultural Chemistry* (2, 1932)
- Gamble, James L, M D, S M 33 Edgehill Rd, Brookline, Mass *Professor of Pediatrics, Harvard Medical School* (2, 1922, 5, 1933)
- Gantt, W Horsley, M D Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md *Associate in Psychiatry* (1, 1935)
- Garbat, Abraham L, M D 103 E 78th St, New York City *Director, Med Service, Lenox Hill Hospital, Clinical Professor of Med, New York Univ Med School* (6, 1913)
- Garner, Raymond L, Ph D University of Michigan Med Sch, Ann Arbor, Mich *Asst Prof of Biological Chemistry* (2, 1947)
- Garrey, Walter Eugene, Ph D, M D Vanderbilt University School of Medicine, Nashville, Tenn *Professor Emeritus of Physiology* (1R, 1910, 2, 1906)
- Gasser, Herbert S, A M, M D, Sc D (hon) Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Director of Laboratories, Member of the National Academy of Sciences* (1, 1915, 3, 1924)
- Gassner, Frank A, M S, D V M Colorado A and M College, Fort Collins *Associate Professor of Physiology, Colorado State College, Associate Pathologist, Colorado State Experiment Station* (1, 1947)
- Gates, Olive, M D 25 Shattuck St, Boston, Mass *Associate Pathologist* (4, 1940)
- Gaunt, Robert, Ph D Syracuse University, Syracuse, N Y *Professor and Chairman of the Department of Zoology* (1, 1939)
- Gay, Leslie N, M D 1114 St Paul St, Baltimore, Md *Director of the Allergy Clinic, Johns Hopkins Hospital, Visiting Physician to Johns Hopkins Hospital, Associate in Medicine, Johns Hopkins University* (6, 1927)
- Geiling, E M K, M S, M D, Ph D University of Chicago, Chicago, Ill *Frank P Hixon Distinguished Service Professor of Pharmacology and Chairman of Department* (1, 1933, 2, 1927, 3, 1925)
- Gulfan, Samuel, Ph D Yale University School

- of Medicine, 333 Cedar St, New Haven 11, Conn  
*Assistant Professor of Physiology* (1, 1930)
- Gellhorn, Alfred, M D** Dept of Pharmacology, College of Physicians and Surgeons, 630 West 168th St, New York, 32, N Y *Associate Professor of Pharmacology* (3, 1946)
- Gellhorn, Ernst, M D**, Ph D Room 116, Medical Sciences, University of Minnesota, Minneapolis *Professor of Neurophysiology* (1, 1930)
- Gemmell, Chalmers L**, M D Department of Pharmacology, Medical School, University of Virginia, Charlottesville *Professor of Pharmacology* (1, 1928, 2, 1935, 3, 1946)
- Gerard, R W**, Ph D, M D University of Chicago, Chicago, Ill *Professor of Physiology* (1, 1927)
- Gersh, Isadore, Ph D** College of Medicine, University of Illinois, 1853 Polk St, Chicago 12, Ill *Associate Professor of Pathology* (1, 1947)
- Gerstenberger, Henry John, M D** Western Reserve University, Cleveland, O *Professor Emeritus of Pediatrics, School of Medicine, Western Reserve University, Director of Pediatrics, Babies and Children's Hospital* (5, 1938)
- Gesell, Robert, M D** University of Michigan, Ann Arbor *Professor of Physiology* (1, 1913)
- Gettler, Alexander O**, A M, Ph D, LL D New York University, 29 Washington Place, New York City *Professor of Chemistry and Toxicology, Toxicologist to Chief Medical Examiner's Office* (2, 1916)
- Gey, George Otto, M D** Johns Hopkins University, Baltimore, Md *Instructor in Surgery* (1, 1940)
- Gibbard, J**, M Sc Laboratory of Hygiene, Dept of Nat Health and Welfare, Ottawa, Canada *Chief, Laboratory of Hygiene* (6, 1916)
- Gibbs, Frederick Andrews, M D** 720 N Michigan Ave, Suite 610, Chicago, Ill (1, 1935)
- Gibbs, Owen Stanley, M B**, Ch B (Edin), M D P O Box 166, Whitehaven, Tenn (1, 1935, 3, 1930)
- Gibson, Robert Banks, Ph D** University Hospital, Iowa City, Iowa *Associate Professor of Biochemistry, State University of Iowa* (1, 1907, 2, 1906)
- Gies, William John, Ph D**, Sc D, LL D, F A C D 632 W 168th St, New York City *Professor of Biological Chemistry, Columbia University* (1R, 1898, 2, 1906, 3R, 1909)
- Gilbert, Ruth, A M**, M D R F D 2, Altamont, N Y *Asst Director, Diagnostic Labs, New York State Department of Health, Albany* (6, 1920)
- Gilman, Alfred, Ph D** College of Physicians and Surgeons, 630 West 168th St, New York 32, N Y *Professor of Pharmacology* (1, 1935, 3, 1934)
- Gilson, Arthur S**, Jr, A M, Ph D Washington University Medical School, St Louis, Mo *Associate Professor of Physiology* (1, 1927)
- Givens, Maurice H**, Ph D Box 5116, Biltmore, North Carolina (1, 1917, 2, 1915)
- Gjessing, Erlend C**, Ph D Univ of Virginia, Charlottesville, Va *1st Professor of Biochemistry* (2, 1918)
- Glass, Howard G**, M S, Ph D Department of Pharmacology, Marquette University School of Medicine, 561 N 15th Street, Milwaukee 3, Wis *Instructor* (3, 1917)
- Glazko, Anthony J**, Ph D Detroit 32, Mich Research Laboratories, Parke, Davis & Co *Research Biochemist* (1, 1912)
- Glick, David, Ph D** University of Minnesota, Minneapolis, Minn *Assoc Prof of Physiological Chemistry* (2, 1936)
- Glickman, Nathaniel, M S** Department of Medicine, University of Illinois College of Medicine, 1853 W Polk St, Chicago 12, Ill *1st Prof of Med and Res Physiologist* (1, 1917)
- Goebel, Walther F**, Ph D The Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Member* (2, 1929, 6, 1937)
- Gaerner, Alfred, Ph G**, Pharm D, M D 366 Sterling Place, Brooklyn, N Y *Assistant Clinical Professor of Medicine Long Island College of Medicine* (2, 1939)
- Goettsch, Marianne, Ph D** School of Tropical Medicine of Columbia University, San Juan, Puerto Rico *Assistant Professor of Chemistry* (2, 1933, 3, 1911)
- Goetzl, Franz R**, Ph D, M D Department of Medical Research, The Permanente Foundation, Oakland 11, Calif *Director* (1, 1947)
- Gold, Harry, M D** Cornell University Medical College, New York City *Prof of Clin Pharmacology, Attending Physician in Charge, Cardiovascular Research Unit, Beth Israel Hospital, Attending Cardiologist, Hospital for Joint Diseases, Visiting Cardiologist, Seaview Hospital, New York City* (3, 1927)
- Goldblatt, Harry, M D** Director Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif (1, 1945, 4, 1927)
- Golden, Alfred, M D** University of Tennessee, Memphis, Tenn *Associate Professor of Pathology and Bacteriology* (4, 1947)
- Goldfarb, Walter, M D** 25 East 86th St, New York City (1, 1938)
- Goldforb, A J**, Ph D College of the City of New York, New York City *Professor of Biology* (1, 1930)
- Goldie, Horace, M D**, D T M Chemotherapy Section, National Cancer Institute, Bethesda, Md (6, 1943)
- Goldring, William, M D** New York University College of Medicine, 477 First Ave, New York

- City Associate Professor of Medicine (1, 1939)
- Goldschmidt, Samuel, Ph D** University of Pennsylvania Medical School, Philadelphia Associate Professor of Physiology (1, 1919, 2, 1915)
- Goldsmith, Grace A M D** Tulane University of Louisiana, New Orleans Assistant Professor of Medicine (5, 1943)
- Goldstein, Avram, M D** Harvard Medical School, Boston, Mass Instructor in Pharmacology (3, 1948)
- Golub, Orville Joseph, M S, Ph D** Bio Service Labs, Inc, 10717 Venice Blvd, Los Angeles 24, Calif Associate (6, 1944)
- Gomori, George, M D** Univ of Chicago, Chicago, Ill Assoc Professor of Medicine (4, 1948)
- Goodman, Louis Sanford, M S, M D** University of Utah School of Medicine, Salt Lake City Professor of Pharmacology and Chairman of the Department of Pharmacology (1, 1946, 3, 1937)
- Goodner, Kenneth, Ph D** Jefferson Medical College, Philadelphia, Pa Professor of Bacteriology (6, 1932)
- Goodpasture, Ernest William, M D** Vanderbilt University Medical School, Nashville, Tenn Professor of Pathology and Dean (4, 1923)
- Gordon, Albert S, M S, Ph D** Washington Square College of Arts and Sciences, New York University, New York City Associate Professor of Biology (1, 1942)
- Gordon, Francis B, M D** University of Chicago 5724 Ellis Avenue, Chicago, Ill Associate Professor of Bacteriology (4, 1947)
- Gordon, Harry H, M D** 4200 E 9th Ave, Denver, Colo Professor of Pediatrics, University of Colorado Medical School, Pediatrician in Chief, Colorado General Hospital (5, 1940)
- Gordon, Irving, M D** Division of Laboratories & Research, N Y State Dept of Health, New Scotland Ave, Albany 1, N Y Assoc Medical Bacteriologist, Assoc Professor of Medicine and of Bacteriology, Albany Medical College (6, 1943)
- Gordon, William G, M A, Ph D** Eastern Regional Research Laboratory, U S Department of Agriculture, Philadelphia 18, Pa Senior Chemist (2, 1939)
- Gortner, Ross Aiken, Jr, M S, Ph D** Shanklin Laboratory, Wesleyan University, Middletown, Conn Associate Professor of Biochemistry (5, 1947)
- Gortner, Willis A, Ph D** Pineapple Research Institute of Hawaii, Honolulu, T H, Head, Dept of Chemistry (2, 1947)
- Goss, Harold, Ph D** University of California College of Agriculture, Davis Professor of Animal Husbandry (2, 1936, 5, 1933)
- Goth, Andres, M D** Southwestern Medical College, 2211 Oak Lawn Avenue, Dallas 4, Tex Associate Professor of Pharmacology (3, 1947)
- Gottschall, Russell Y, M S, Ph D** Bureau of Laboratories, Michigan Department of Health, Lansing Bacteriologist (6, 1939)
- Goudsmit, Arnoldus, Jr, M D, Ph D** 4141 Windsor Rd, Youngstown 7, Ohio (1, 1940)
- Govier, William M, M D** The Upjohn Co, Kalamazoo 99, Mich Research Division (3, 1944)
- Grabfield, G Philip, M D** 27 Forest St, Milton, Mass Associate in Medicine and Pharmacology, Harvard Medical School (At present on leave of absence, Col M C, U S A) (3, 1923)
- Grady, Hugh G, M D** Army Institute of Pathology, Washington, D C Pathologist (4, 1940)
- Graef, Irving, M D** New York University College of Medicine, New York City Assistant Professor of Clin Medicine (4, 1941)
- Graham, Claire E, Ph D** Wilson Laboratories, 4221 S Western Ave, Chicago, Ill Director of Research (2, 1948)
- Graham, Clarence H, Ph D** Columbia University, New York 27, N Y Professor of Psychology (1 1933)
- Graham, Helen Tredway, A M, Ph D** Euclid Ave and Kingshighway, St Louis, Mo Associate Professor of Pharmacology, Washington University School of Medicine (1, 1933, 3, 1931)
- Grant, R Lorimer, M S, Ph D** Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D C Pharmacologist (2, 1938)
- Graubard, Mark, M A, Ph D** Dept of Physiology, University of Minnesota, Minneapolis (1, 1940)
- Grauer, Robert C, M D** Allegheny General Hospital, Pittsburgh Pa Director of Research, and Head of Dept of Endocrinology and Metabolism, William H Singer Memorial Research Laboratory, Assistant Professor of Medicine, School of Medicine, University of Pittsburgh (4, 1941)
- Gray, John S, M S, Ph D M D** Northwestern Univ Medical School, 303 L Chicago Ave, Chicago 11, Ill Professor of Physiology and Chairman of Department (1, 1937)
- Gray, M Geneva, M A, Ph D** Laboratories of Arthur D Little, Inc, Cambridge, Mass Director Pharmacological Research (3, 1946)
- Gray, Samuel H, M D** The Jewish Hospital of St Louis, Kingshighway and Forest Park Blvd, St Louis, Mo Director of Laboratories, Jewish Hospital, Associate Professor of Pathology, Washington University (4, 1939)
- Gray, Stephen W, Ph D** Emory Univ Sch of

- Med, Emory Univ, Ga *Asst Professor of Anatomy* (1, 1948)
- Graybiel, Ashton, M D** U S Naval Sch of Aviation Med and Res, Naval Air Station, Pensacola, Fla *Coordinator of Research* (1, 1948)
- Greaves, J D**, M S, Ph D Western Regional Research Lab, U S Dept of Agriculture, 800 Buchanan St, Albany 6, Calif *Biochemist* (2, 1938)
- Greaves, Joseph E**, Ph D 3211 S W 10 Ave, Portland, Ore (2, 1940)
- Greeley, Paul O**, A M, Ph D, M D University of Southern California Medical School, University Park, Los Angeles *Dept of Aviation Medicine* (1, 1940)
- Green, Arda Alden, M D** Cleveland Clinic, Euclid and E 93rd St, Cleveland 6, O *Research Division* (2, 1932)
- Green, Daniel M**, M D, M S Univ of Washington Sch of Med, Seattle, Wash *Assoc Prof of Medicine* (1, 1948, 3, 1942)
- Green, David E**, Ph D University of Wisconsin, Enzyme Institute, Madison, Wis *Prof of Enzyme Chemistry* (2, 1941)
- Green, Harold David, M D** Bowman Gray School of Medicine, Wake Forest College, Winston-Salem 7, N C *Professor of Physiology and Pharmacology* (1, 1936, 3, 1945)
- Greenberg, David Morris, Ph D** University of California, Berkeley *Professor and Chairman of Division of Biochemistry* (2, 1927, 5, 1946)
- Greenberg, Louis D**, Ph D Univ of Calif Medical Center, 3rd and Parnassus Aves, San Francisco 22, Calif *Assistant Professor of Pathology, Univ of California Med School* (2, 1946)
- Greene, Carl Hartley, Ph D**, M D 140 E 54th St, New York City *Associate Professor of Clinical Medicine, New York Post Graduate Medical School of Columbia University, Clinical Professor of Medicine, Long Island College of Medicine* (1, 1921, 2, 1922, 4, 1924)
- Greene, Harry S N**, M D, C M Department of Pathology, Yale University School of Medicine, New Haven, Conn *Professor of Pathology* (4, 1937)
- Greene, James Alexander, M D** Baylor University, College of Medicine, Buffalo Drive, Houston, Texas *Professor and Chairman of the Department of Internal Medicine and Dean of the Clinical Faculty* (1, 1939)
- Greene, Ronald R**, M S, M D Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill *Instructor in Physiology, Instructor in Obstetrics and Gynecology* (1, 1941)
- Greengard, Harry, Ph D**, M D Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill *Assistant Professor of Physiology* (1, 1939)
- Greenstein, Jesse P**, Ph D National Cancer Institute, Bethesda, Md *Chief Biochemist* (2, 1935)
- Greenwald, Isidor, Ph D** 477 First Ave, New York City *Associate Professor of Chemistry, New York University College of Medicine* (2, 1912, 5, 1933)
- Greep, Roy O**, Ph D Harvard School of Dental Medicine, 188 Longwood Ave, Boston 15, Mass *Assistant Professor of Dental Science (Dental Medicine), Teaching Fellow in Anatomy (Medical School)* (1, 1940)
- Greer, C M**, M S Vanderbilt University School of Medicine, Nashville, Tenn *Research Associate in Pharmacology* (3, 1938)
- Gregersen, Magnus I**, A M, Ph D College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York City *Professor of Physiology* (1, 1933)
- Gregg, Donald Eaton, M S**, Ph D, M D Armored Medical Research Laboratory, Fort Knox, Ky *Chief Research Physician* (1, 1933)
- Gregory, John E** Hahnemann Medical College, Philadelphia, Pa *Professor of Pathology* (6, 1948)
- Gregory, Raymond L**, Ph D, M D University of Texas School of Medicine, 1419—24th St, Galveston *Professor of Medicine* (1, 1945)
- Greig, Margaret E**, B A, M A, Ph D Vanderbilt Univ School of Medicine, Nashville 4, Tenn *Assistant Professor in Pharmacology* (3, 1946)
- Greisheimer, Esther M**, Ph D, M D Temple University Medical School, 3400 N Broad St, Philadelphia, Pa *Professor of Physiology* (1, 1925)
- Grenell, Robert G**, Ph D University of Pennsylvania Eldridge Reeves Foundation, Philadelphia, Pa *Senior Fellow, USPHS, Natl Inst of Health* (1, 1945)
- Griffin, Angus, Ph D** Department of Bacteriology, George Washington University School of Medicine, 1335 H St, N W, Washington, D C *Associate Professor of Bacteriology* (6, 1940)
- Griffith, Fred R, Jr**, M A, Ph D 24 High St, Buffalo, N Y *Professor of Physiology, University of Buffalo Medical School* (1, 1923, 5, 1933)
- Griffith, Wendell H**, M S, Ph D University of Texas Medical School, Galveston, Texas *Prof and Chn, Dept of Biochemistry and Nutrition* (2, 1923, 5, 1934)
- Grimson, Keith S**, M D Duke University School of Medicine, Durham, N C *Associate Professor of Surgery* (1, 1943)
- Grindlay, John H**, M D Mayo Clinic, Rochester, Minn (1, 1945)
- Groat, Richard A**, Ph D Bowman Gray School of

- Medicine, Winston Salem 7, N C *Assoc Professor of Anatomy* (1, 1945)
- Groat, William A, M D 713 E Genesee St, Syracuse, N Y *Professor of Clinical Pathology, Syracuse University College of Medicine* (6, 1917)
- Grodins, Fred S, Ph D, M D Northwestern University Medical School, 303 E Chicago Ave, Chicago 11, Ill *Associate Professor of Physiology* (1, 1945)
- Grollman, Arthur, M D, Ph D Southwestern Medical College, 2211 Oak Lawn Ave, Dallas, Texas *Professor of Medicine and Chairman of the Department of Experimental Medicine, Professor of Pharmacology and Chairman of the Department of Physiology and Pharmacology* (1, 1928, 3, 1933)
- Gross, Erwin G, Ph D, M D Medical Laboratories, State University of Iowa, Iowa City *Professor of Pharmacology* (1, 1927, 2, 1923, 3, 1927)
- Gross, Robert E, M D Harvard Medical School, 300 Longwood Ave, Boston, Mass *Ladd Professor of Children's Surgery* (4, 1940)
- Grossman, Morton Irvin, M S, Ph D, M D, University of Illinois College of Medicine, Chicago 12 *Assoc Professor of Physiology* (1, 1946)
- Groupe, Vincent Ph D Univ of Connecticut, Storrs Agric Exper Station, Dept of Animal Diseases Storrs, Conn *Assoc Professor in Animal Diseases* (6 1946)
- Gruber, Charles M, A M, M D, Ph D Jefferson Medical College, 1025 Walnut St, Philadelphia, Pa *Professor of Pharmacology* (1, 1914, 3, 1919)
- Gruber, Charles M, Jr, M D Jefferson Medical College Hospital, Philadelphia, Pa *Research Fellow in Hematology* (3, 1948)
- Gruhzit, Oswald M, M D Research Laboratories, Parke, Davis & Co, Detroit, Mich *Research in Pathology and Pharmacology* (4, 1928)
- Grundfest, Harry, A M, Ph D Columbia Univ P and S, 630 West 168th St, New York 32, N Y *Associate in Neurology* (1, 1932)
- Gudernatsch, F, Ph D 41 Fifth Avenue, New York 3, N Y (1, 1930)
- Guerra, Francisco, M Sc, Med Major, B S and Litt M D Facultad de Medicina, Universidad Nacional de Mexico, Mexico D F *Professor of Pharmacology* (3, 1947)
- Guerrant, N B, M S, Ph D Pennsylvania State College, State College *Professor of Biological Chemistry* (2, 1934, 5, 1933)
- Guest, George Martin, M S, M D Children's Hospital, Cincinnati, Ohio *Prof of Pediatrics, Univ of Cincinnati College of Medicine and Graduate School* (2, 1933)
- Guest, Maurice Mason, Ph D Dept of Physiology, Wayne Univ, College of Medicine, Detroit 26, Mich *Associate Professor of Physiology* (1, 1946)
- Gulick, Addison, A M, Ph D 308 Westmount Ave, Columbia, Mo *Professor of Physiological Chemistry, University of Missouri* (1, 1915, 5, 1933)
- Gunn, Francis D, M D, Ph D University of Utah, School of Medicine, Salt Lake City *Professor and Head of Dept of Pathology* (4, 1938)
- Gunsalus, Irwin C, Ph D Indiana University, Bloomington *Professor of Bacteriology* (2, 1946)
- Gurin, Samuel, Ph D University of Pennsylvania School of Medicine, Philadelphia *Professor of Physiological Chemistry* (2, 1938)
- Gustavson, R G, Ph D University of Nebraska, Lincoln *Chancellor* (2, 1927)
- Gustus, Edwin L, Ph D Bjorksten Research Lab, 185 N Wabash Ave, Chicago, Ill *Vice President* (2, 1934)
- Guthrie, Charles Claude, M D, Ph D, Sc D University of Pittsburgh Medical School, Pittsburgh, Pa *Professor of Physiology and Pharmacology* (1, 1905, 3, 1909)
- Gutierrez-Noriega, Carlos, M D Facultad de Medicina, Av Grau Lima, Peru *Professor of Pharmacology* (3, 1948)
- Gutman, Alexander B, M D Columbia Research Service, Welfare Island, New York 17 *Director* (2, 1947)
- Guttman, Rita M, M A, Ph D Brooklyn College, Brooklyn, N Y *Instructor in Physiology* (1, 1946)
- Gyorgy, Paul, M D University Hospital, Philadelphia, Pa *Prof of Clin Pediatrics* (2, 1938, 5, 1939)
- Haag, Harvey B, M D Medical College of Virginia, Richmond *Professor of Pharmacology* (3, 1934)
- Haag, J R, Ph D Oregon Agricultural Experiment Station, Corvallis *Chemist* (2, 1947, 5, 1941)
- Haas, Erwin, Ph D Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles 27, Calif *Research Associate* (2, 1946)
- Haberman, Sol, M A, Ph D Wm Buchanan Blood, Plasma and Serum Center, Baylor Hospital, Dallas, Texas *Chief of Bacteriology and Serology Services* (6, 1944)
- Hadidian, Zareh, Ph D Tufts College, Medical School, Boston, Mass (1, 1945)
- Hadley, Philip Bardwell, Ph D Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh *Chief of Bacteriological Service and Research Bacteriologist* (4, 1927)
- Hafkesbring, H Roberta, Ph D Woman's Medi-

- cal College of Pennsylvania, East Falls, Philadelphia *Professor of Physiology* (1, 1931)
- Haggard, Howard W, M D 4 Hillhouse Ave, New Haven, Conn *Director of the Laboratory of Applied Physiology, Yale University* (1, 1919, 2, 1920)
- Hahn, Paul F, Ph D Meharry Medical College, Nashville, Tenn *Director of Cancer Research Laboratories* (1, 1946, 4, 1939)
- Haig, Charles, M A, Ph D New York Medical College, Fifth Ave at 105th St, New York City *Associate Professor of Physiology and Biochemistry* (1, 1942)
- Haist, Reginald E, M A, M D, Ph D University of Toronto, Toronto, Ontario, Canada *Associate Professor of Physiology* (1, 1943)
- Halbert, Seymour P, M D School of Public Health, University of North Carolina, Chapel Hill, N C *Assistant Professor, Experimental Medicine* (6, 1947)
- Haldi, John, A M, Ph D Emory University, Emory University, Ga (1, 1928)
- Hale, Wm M, M D The State University of Iowa College of Medicine, Iowa City *Professor and Head of Dept of Bacteriology* (1, 1941, 6, 1935)
- Hall, F G, M A, Ph D Duke Univ School of Medicine, Dept of Physiology and Pharmacology, Durham, N C (1, 1937)
- Hall, George Edward, M D, Ph D University of Western Ontario, Ottawa Ave and Waterloo St, London, Canada *Dean of the Faculty of Medicine* (1, 1938)
- Hall, Victor E, M A, M D Department of Physiology, Stanford University, Calif *Professor of Physiology* (1, 1934)
- Hall, W Knowlton, Ph D Univ of Georgia School of Medicine, Augusta, Ga *Associate Professor of Biochemistry* (2, 1948)
- Hallenbeck, George Aaron, Ph D, M D Mayo Clinic, Rochester, Minn *Research Associate* (1, 1946)
- Halliday, Nellie, Ph D Lugnut Honda Home, Laboratory of Experimental Oncology, San Francisco, Calif (5, 1933)
- Halpert, Béla, M D University of Oklahoma School of Medicine, Oklahoma City *Director of Laboratories and Professor of Clinical Pathology* (4, 1936)
- Halstead, Ward C, M A, Ph D Dept of Medicine, University of Chicago, Chicago, Ill *Associate Professor Experimental Psychology, Division of Psychiatry* (1, 1942)
- Ham, Arthur W, M B University of Toronto, Toronto 5, Canada *Professor of Anatomy, in charge of Histology* (4, 1939)
- Hambourger, Walter E, Ph D, M D G D Searle & Co, P O Box 5110, Chicago, Ill *Chief Pharmacologist* (3, 1934)
- Hamilton, Bengt L K, M D U S Marine Hospital, Staten Island 4, N Y *Medical Director, U S Public Health Service* (2, 1925)
- Hamilton, James B, Ph D Department of Anatomy, Long Island College of Med, 350 Henry Street, Brooklyn 2, N Y (1, 1938)
- Hamilton, Paul B, M A, Ph D Alfred I Du Pont Institute, Nemours Foundation, Rockland Rd, Wilmington 99, Del *Chief of Biochemistry* (2, 1946)
- Hamilton, Tom S, M S, Ph D University of Illinois, Urbana *Professor and Chief in Animal Nutrition* (2, 1937, 5, 1938)
- Hamilton, W F, Ph D University of Georgia School of Medicine, Augusta *Professor of Physiology and Pharmacology* (1, 1924)
- Hammett, Frederick S, M S, A M, Ph D 493 Commercial St, Provincetown, Mass (19, 1920, 2, 1917)
- Hammon, William McD, M D, M P H, Ph D University of California School of Public Health, San Francisco, Calif *Professor of Epidemiology, George Williams Hooper Foundation, Professor of Epidemiology* (1, 1944)
- Hampel, C W, Ph D New York University College of Medicine, New York, N Y *Visiting Professor of Physiology and Anatomy* (1, 1936)
- Hamre, Dorothy, Ph D Squibb Institute for Medical Research, New Brunswick, N J *Research Associate* (6, 1948)
- Hand, David B, Ph D New York State Agricultural Experiment Station, Geneva, New York *Head, Division of Food Science and Technology and Prof of Biochemistry* (2, 1947)
- Handler, Philip, M S, Ph D Duke University School of Medicine, Durham, N C *Associate Professor of Biochemistry and Nutrition* (2, 1944, 5, 1946)
- Handler, Carroll A, Ph D Baylor Univ College of Medicine, Houston 1, Texas *Professor of Physiology and Pharmacology* (3, 1942)
- Haney, Hance F, Ph D, M D University of Oregon Medical School, Portland *Assistant Professor of Medicine* (1, 1939)
- Hanger, Franklin, M D College of Physicians and Surgeons, 630 W 168th St, New York City *Associate Professor of Medicine, Columbia University* (6, 1930)
- Hanke, Martin E, Ph D University of Chicago, Chicago, Ill *Associate Professor of Biochemistry* (2, 1925)
- Hanke, Milton Theo, Ph D 7550 S Green St, Chicago, Ill *Research Consultant, Biochemistry and Nutrition* (2, 1919)
- Hanks, John H, Ph D Harvard University Medical School, Boston, Mass *Dept of Bacteriology, Bacteriologist of the Leonard Wood Memorial* (6, 1935)

- Hansen, Arild E, M D University of Texas Medical School, Galveston *Professor of Pediatrics and Chairman of the Department, Director of the University of the Texas Child Health Program* (4, 1941, 5, 1942)
- Hanzal, Ramon F, M A, Ph D Killian Research Laboratories, 49 W 45th St, New York City *Biochemist* (2, 1935)
- Hanzlik, Paul J, M D School of Medicine, Stanford University, Sacramento and Webster Sts, San Francisco, Calif *Professor of Pharmacology* (1, 1912, 2, 1919, 3, 1912)
- Hardy, James Daniel, A M, Ph D Russell Sage Institute of Pathology, 525 E 68th St, New York City *Research Associate* (1 1939)
- Hardy, Mary, D Sc The Brearley School, 610 E 83rd St, New York City *Teacher of Science* (1, 1933)
- Hare, Kendrick, Ph D 1300 York Ave, New York, N Y (1, 1938)
- Harford, Carl G, M D Washington Univ Sch of Med, St Louis, Mo *Asst Professor of Med and Preventive Med* (6, 1948)
- Harger, R N, M A, Ph D Indiana University School of Medicine, Indianapolis *Professor of Biochemistry and Toxicology* (2, 1938)
- Harkins, Henry Nelson, M S, Ph D, M D Johns Hopkins Hospital, Baltimore, Md *Associate Professor of Surgery, Johns Hopkins University Medical School* (1, 1942)
- Harmon, Paul M, A M, Ph D Indiana University, Bloomington *Professor of Physiology* (1, 1930)
- Harne, O G University of Maryland School of Medicine, Baltimore *Associate Professor of Histology* (1, 1935)
- Harned, Ben King, M S, Ph D Lederle Laboratories, Pearl River, N Y *Assoc Director, Pharmacology Research* (2, 1931, 3, 1941)
- Harris, Albert H, M D N Y State Dept of Health, Division of Laboratories and Research, New Scotland Ave, Albany 1, N Y *Asst Director for Local Labs* (6, 1937)
- Harris, Albert Sidney, Ph D College of Medicine, Baylor University, Houston 5, Texas (1, 1939)
- Harris, Milton, Ph D Harris Research Laboratories, 1246 Taylor St, N W, Washington 11, D C *President* (2, 1939)
- Harris, Paul N, M D The Eli Lilly and Company, Indianapolis, Ind *Pathologist, Division of Pharmacology* (3, 1948)
- Harris, Philip L, M S, Ph D Distillation Products Inc, 755 Ridge Road W, Rochester 13, N Y *Head of Biochemistry Research Department, Associate in Physiology, Univ of Rochester Sch of Med and Dentistry* (5, 1945, 2, 1946)
- Harris, Robert S Massachusetts Institute of Technology, Cambridge *Professor of Nutritional Biochemistry* (5, 1941)
- Harris, S C, M S, Ph D Department of Physiology and Pharmacology, Northwestern University, Chicago, Ill *Chairman of Department* (1, 1947)
- Harris, T N, M D Children's Hospital of Philadelphia, Philadelphia, Pa *Associate in Pediatrics, Univ of Pennsylvania* (6, 1946)
- Harris, William H, M D Tulane University School of Medicine, New Orleans, La *Assistant Professor of Pathology and Bacteriology* (4, 1925)
- Harrison, Frank, M S, Ph D University of Tennessee College of Medicine, Memphis *Professor and Chief, Division of Anatomy* (1, 1941)
- Harrison, James A, Ph D Temple Univ, Philadelphia 22, Pa *Professor of Biology* (6, 1946)
- Harrison, Ross Granville, M D, Ph D, Sc D Osborn Zoological Laboratory, New Haven, Conn *Sterling Professor of Biology, Emeritus, Yale University, Chairman of the National Research Council, Member of the National Academy of Sciences* (1, 1906)
- Harrison, R Wendell, M D, Ph D Ellis Ave at 58th St, Chicago 37, Ill *Professor of Bacteriology, Dean of Faculties and Vice President, Univ of Chicago* (6, 1934)
- Harrow, Benjamin, M A, Ph D College of the City of New York, Convent Ave and 139th St, New York City *Professor of Chemistry* (2, 1927)
- Hart, E B, B S University of Wisconsin Agricultural College, Madison, Wis *Professor of Biochemistry, University of Wisconsin* (2, 1910, 5, 1933)
- Hart, E Ross, M S, Ph D Jefferson Medical College, 1025 Walnut St, Philadelphia, Pa *Assistant Professor of Pharmacology* (3, 1944)
- Hart, William M, Ph D Temple Medical School, Broad and Ontario Sts, Philadelphia 40, Pa *Assistant Professor of Physiological Chemistry* (1, 1945)
- Hartline, H K, M D Johnson Foundation University of Pennsylvania Hospital, Philadelphia 4, Pa, *Assistant Professor of Biophysics* (1, 1929)
- Hartman, Carl G, A M, Ph D Department of Zoology, University of Illinois, Urbana *Professor of Zoology and Head of the Department, Member, National Academy of Sciences* (1, 1921)
- Hartman, Frank Alexander, A M, Ph D Department of Physiology, Ohio State University, Columbus *Professor of Physiology and Chairman of the Department* (1, 1916)
- Hartman, F W, M D Henry Ford Hospital, Detroit, Mich *Pathologist* (4, 1927)
- Hartmann, Alexis F, M S, M D 500 S Kingshighway, St Louis, Mo *Professor of Pediatrics,*

- Washington University School of Medicine (2, 1932)
- Harvey, A McGhee, A B, M D Johns Hopkins Hospital, Baltimore 5, Md *Professor of Medicine, Johns Hopkins Univ Medical School, Physician-in-Chief, Johns Hopkins Hospital* (1, 1916, 3, 1946)
- Harvey, E Newton, Ph D Guyot Hall, Princeton, N J *Henry Fairfield Osborn Professor of Biology, Princeton University, Member, National Academy of Sciences* (1, 1914, 2, 1916)
- Hass, George, M D Presbyterian Hospital of Chicago, Chicago, Ill *Chairman, Dept of Pathology, University of Illinois, College of Medicine, Chicago Professor of Pathology* (4, 1939)
- Hassid, William Z, M S, Ph D Division of Plant Nutrition, Univ of California, Berkeley, Calif *Professor of Plant Nutrition* (2, 1916)
- Hastings, A Baird, Ph D, Sc D Harvard Medical School, Boston, Mass *Hamilton Kuhn Professor of Biological Chemistry, Member, National Academy of Sciences* (1, 1927, 2, 1921, 5, 1940)
- Hathaway, Milicent L, M A, Ph D Bureau of Human Nutrition and Home Economics, (Food and Nutrition Division), Agricultural Research Administration, Washington 25, D C (5, 1945)
- Hauck, Hazel M, Ph D Cornell University, Ithaca, N Y *Professor of Home Economics* (5, 1941)
- Hauge, Siegfred M, Ph D Purdue University Agricultural Experiment Station, Lafayette, Ind *Research Associate in Biochemistry* (5, 1933)
- Haurowitz, Felix, M D, D Sc Indiana Univ, Bloomington, Ind *Professor of Biochemistry* (6, 1948)
- Haury, Victor G, M B, M D Box 206, Wellsville, Kansas (3, 1939)
- Haven, Frances L, M A, Ph D University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y *Associate in Biochemistry* (2, 1941)
- Hawk, Philip B, M S, Ph D Food, Drug and Cosmetic Consultants, 30 Rockefeller Plaza, Room 4631, New York City *President* (1, 1903, 2, 1906)
- Hawkins, J E, Jr, B A (Oxon), Ph D Merck Institute for Therapeutic Research, Rahway, N J *Physiologist* (1, 1943)
- Hawkins, William Bruce, M D University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y *Associate Professor of Pathology* (4, 1933)
- Hawley, Estelle E, Ph D Medical School, University of Rochester, Rochester, N Y *Research Fellow in Pediatrics* (5, 1935)
- Hawn, Clinton van Zandt, M D Mary Imogene Bassett Hospital, Cooperstown, N Y *Pathologist, Director of Otsego County Laboratories* (4, 1918)
- Hay, Eleanor Clarke, Ph D 7 Greenhill Rd, Madison, N J (1, 1915)
- Hayman, J M, Jr, M D Lakeside Hospital, Cleveland, O *Professor of Clinical Medicine and Therapeutics, Western Reserve University* (1, 1928, 3, 1932)
- Haynes, Florence W, M A, Ph D Harvard Medical School, 25 Shattuck St, Boston, Mass *Research Associate in Medicine* (1, 1937)
- Hays, Edwin Everett, M S, Ph D The Armour Laboratories, Chicago, Ill *Director of Biochemical Research* (2, 1946)
- Haythorn, Samuel R, M D Allegheny General Hospital, 320 E North Ave, Pittsburgh, Pa *Director of William H Singer Memorial Laboratory* (1, 1925)
- Haywood, Charlotte, M, Ph D Mount Holyoke College, South Hadley, Mass *Professor of Physiology* (1, 1939)
- Hazen, Elizabeth L, M A, Ph D New York State Department of Health Laboratories, 339 E 25th St, New York City *Senior Bacteriologist* (6, 1931)
- Hazleton, Lloyd W, Ph D Box 333, Falls Church, Va *Research Consultant* (3, 1914)
- Hechter, Oscar M, Ph D Worcester Foundation Exper Biol, 222 Maple Ave, Shrewsbury, Mass (1, 1945)
- Hegnauer, Albert H, Ph D Boston University School of Medicine, Boston, Mass *Associate Professor of Physiology* (1, 1937)
- Hegsted, David Mark, M S, Ph D Harvard School of Public Health, Boston, Mass *Professor of Nutrition* (5, 1944)
- Heidelberger, Michael, Ph D 620 W 168th St, New York City *Prof of Immunochemistry, Columbia University, Chemist to the Presbyterian Hospital* (2, 1927, 6, 1935)
- Heilbrunn, Lewis Victor, Ph D University of Pennsylvania, Philadelphia *Professor of Zoology* (1, 1930)
- Helm, J William, Ph D 1 Yale Ave, Dayton 6, Ohio Aero Medical Laboratory, Army Air Forces, Wright Field *Principal Research Physiologist, Assistant in Physiology, Harvard School of Public Health* (1, 1936)
- Heinbecker, Peter, M D Washington University Medical School, St Louis, Mo *Associate Professor of Clinical Surgery* (1, 1930)
- Hemle, Robert W, M D Western Reserve Univ, Cleveland, Ohio *Asst Professor of Medicine* (5, 1948)
- Helf, O M, M S, Ph D New York University, University Heights, New York City *Associate Professor of Biology* (1, 1932)



- Hellbaum, Arthur A, M A, Ph D, M D University of Oklahoma School of Medicine, Oklahoma City *Professor of Pharmacology* (1, 1937, 3, 1945)
- Hellebrandt, Frances Anna, M D Medical College of Virginia, Richmond *Professor of Physical Medicine* (1, 1933)
- Heller, Carl G, M D, Ph D University of Oregon Medical School, Portland 1 *Associate Professor of Physiology and Medicine* (1, 1945)
- Heller, Victor G, Ph D Oklahoma A & M College, Stillwater *Head of the Department of Agricultural Chemistry Research* (2, 1935, 5, 1935)
- Hellerman, Leslie, Ph D Johns Hopkins University School of Medicine, 710 N Washington St, Baltimore 5, Md *Associate Professor of Physiological Chemistry* (2, 1935)
- Helmer, Oscar Marvin, M S, Ph D Indianapolis General Hospital, Indianapolis, Ind *Head, Dept of Physiological Chem* (2, 1935)
- Hemingway, Allan, Ph D 241 Cecil St, S E, Minneapolis *Associate Professor of Physiology, University of Minnesota* (1, 1933)
- Hendrix, Byron M, Ph D School of Medicine, University of Texas, Galveston *Professor of Biochemistry* (2, 1920)
- Hendrix, James Paisley, B S, M A, M D Duke Hospital, Durham, N C *Associate in Medicine (in charge of Therapeutics), Associate in Physiology and Pharmacology, Duke University School of Medicine* (3, 1942)
- Hendry, Jessie L, M A Division of Laboratories and Research, New York State Department of Health, New Scotland Ave, Albany *Senior Bacteriologist* (6, 1938)
- Henle, Werner, M D 1740 Bainbridge St, Philadelphia 46, Pa *Associate Professor of Virology in Pediatrics* (6, 1938)
- Henry, James P, M A, M Sc, M R C S C R C P Aero Medical Laboratory, Wright Field, Dayton, Ohio On leave of absence from the University of Southern California, Los Angeles, Calif (1, 1947)
- Henschel, Austin F, Ph D University of Minnesota, Minneapolis *Assistant Professor of Physiological Hygiene* (1, 1944)
- Hepburn Joseph Samuel, M A, M S, Ph D M D Chem D 235 N 15th St Philadelphia 2, Pa *Professor of Chemistry and Research Associate in Gastro Enterology Registrar, Hahnemann Medical College and Hospital* (2, 1915)
- Hepler, Opal E, Ph D, M D Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill *Assistant Professor of Pathology* (4, 1939)
- Herbst, Robert M, Ph D Kedzie Chemical Laboratory, Michigan State College, East Lansing *Associate Professor of Chemistry* (2, 1938)
- Herget, Carl M, Ph D Army Chemical Center, Medical Division, Edgewood, Md *Chief, Biophysics Section* (1, 1948)
- Herricks, Julia F, M A, Ph D Mayo Foundation, Rochester, Minn *Associate Professor of Experimental Medicine* (1, 1933)
- Herrin, Raymond C, Ph D, M D University of Wisconsin Medical School, Madison *Associate Professor of Physiology* (1, 1932)
- Herrington, Lovic P, M A, Ph D 290 Congress Ave, New Haven, Conn *Associate Director, John B Pierce Laboratory of Hygiene, Research Associate Professor, Dept of Public Health, Yale Medical School* (1, 1942)
- Herriott, Roger M, Ph D Johns Hopkins School of Hygiene and Public Health, Baltimore, Md *Prof of Biochemistry* (2, 1940)
- Herrmann, George, Ph D, M D University of Texas, Medical Branch, Galveston *Professor of Medicine* (4, 1925)
- Hermann, Julian B, Ph B, M D, 1185 Park Ave, New York City (3, 1941)
- Herrmann, Louis George, M D Cincinnati General Hospital, Cincinnati 29, O *Associate Professor of Surgery, University of Cincinnati College of Medicine, Attending Surgeon, Surgical Services, Cincinnati General Hospital and Children's Hospital and Christian R Holmes Hospital of University of Cincinnati* (4, 1933)
- Hershey, A D, Ph D Washington University School of Medicine, St Louis, Mo *Associate Professor of Bacteriology and Immunology* (6, 1942)
- Hertz Roy, Ph D, M D National Institutes of Health, Bethesda 14, Md *P H Surgeon (R), Division of Physiology* (1 1945)
- Hertz, Saul, M D 330 Brookline Ave, Boston, Mass Beth Israel Hospital *Instructor, Harvard Medical School* (4, 1935)
- Hertzman, Alrick B, Ph D St Louis University School of Medicine, St Louis, Mo *Professor of Physiology and Director of the Department* (1, 1925)
- Herwick, Robert P, Ph D, M D, LL B U S Food and Drug Administration, Washington, D C *Chief, Drug Division, Associate Prof Pharmacology, Georgetown Medical School, Adjunct Clinical Professor Medicine (Therapeutics) George Washington Medical School* (3, 1938)
- Hess, Charles L, M S, M D 308 Davidson Bldg, Bay City, Mich (1, 1916)
- Hess, Walter C, Ph D Georgetown Medical

- School, 39th St and Reservoir Rd, N W, Washington, D C *Professor of Biological Chemistry* (2, 1935)
- Hetherington, Albert W**, M S, Ph D School of Aviation Medicine, Randolph Field, Texas (1, 1943)
- Hewetson, Jean Hawks**, M D Biglerville, Pa (5, 1944)
- Hewitt, Earl Albon**, M S, Ph D Iowa State College, Ames *Associate Professor of Veterinary Physiology* (1, 1932)
- Hewitt, Julia A W**, B A 2631 Central Ave, N E, Minneapolis 13, Minn *Senior Technician, Huntington Hospital Lab, Huntington, N Y* (6, 1921)
- Heyroth, Francis F**, M D, Ph D University of Cincinnati, Cincinnati, Ohio *Assoc Prof of Ind Toxicology and Asst Director, Kettering Lab, Assoc Prof of Biological Chem, College of Med* (2, 1935)
- Hiatt, Edwin P**, M A, Ph D North Carolina University School of Medicine, Chapel Hill *Associate Professor of Physiology* (1, 1942)
- Hickman, Kenneth C D**, Ph D 56 Thackeray Road, Rochester 10, N Y *Consultant, Arthur D Little, Inc* (2, 1941)
- Hiestand, William A**, Ph D Department of Biology, Purdue University, Lafayette, Ind *Professor of Physiology* (1, 1947)
- Higgins, George M**, Ph D Mayo Clinic, Rochester, Minn *Professor of Experimental Biology, Mayo Foundation* (5, 1948)
- Higgins, Harold Leonard**, M D 322 Franklin, Newton, Mass (1, 1914, 5, 1933)
- Highman, Benjamin**, M D National Institutes of Health, Bethesda, Md *Surgeon, Pathology Lab* (4, 1947)
- Hill, Edgar S**, M S, Ph D Washington University, College of Dentistry St Louis, Mo *Associate Professor of Biological Chemistry and Physiology* (2, 1936)
- Hill, Robert M**, M S, Ph D University of Colorado Med Ctr, Denver, Colo *Prof of Biochemistry* (2, 1933)
- Hill, Samuel E**, M A, Ph D 18 Collins Ave, Troy, N Y *Research Worker, The Behr-Manning Corp* (1, 1934)
- Hiller, Alma**, Ph D Presbyterian Hospital of the City of Chicago, Chicago, Ill *Assoc Biochemist* (2, 1929)
- Himwich, Harold E**, M D Fallston, Md *Chief Clinical Research Branch, Medical Division, Army Chemical Center, Maryland* (1, 1925, 5, 1933)
- Himwich, William A**, M S, Ph D Toxicology Section, Medical Division, Army Chemical Center, Md (1, 1947)
- Hine, Charles H**, M A, Ph D, M D Pharmacology and Toxicology Department, Medical Center, The University of California Medical School, San Francisco 22, Calif *Lecturer in Toxicology, University of California Medical School, Consulting Pharmacologist and Toxicologist, Shell Development Co* (3, 1917)
- Hines, Harry M**, M S, Ph D The State University of Iowa, Iowa City *Professor of Physiology* (1, 1928)
- Hines, Marion**, Ph D Department of Anatomy, Emory University, Ga *Professor of Experimental Anatomy* (1, 1932)
- Hinrichs, Marie**, Ph D, M D Southern Illinois Normal University, Carbondale *Professor of Physiology, Director of Student Health Service* (1, 1928)
- Hinsey, Joseph C**, M S, Ph D Cornell University Medical College, 1300 York Ave, New York City *Professor of Anatomy and Dean of the Medical College* (1, 1929)
- Hirschmann, Hans**, M D, Ph D Lakeside Hospital, Cleveland, Ohio *Assistant Professor of Biochemistry, Department of Medicine, Western Reserve University* (2, 1946)
- Hirszfeld, Ludwik**, M D University of Wrocław, Wrocław, Ul Chrobinskiego 4, Poland *Professor of Microbiology, Director of Medical Microbiology* (6, 1916)
- Hisaw, Frederick L**, M S, Ph D The Biological Laboratories, Harvard University, Cambridge, Mass *Professor of Zoology* (1, 1932)
- Hitchcock, David I**, Ph D 333 Cedar St, New Haven, Conn *Associate Professor of Physiology, Yale University* (2, 1930)
- Hitchcock, Fred A**, M Sc, Ph D Ohio State University, Columbus *Professor of Physiology* (1, 1927, 5, 1933)
- Hitchcock, Philip**, M S, Ph D Department of Physiology and Pharmacology, Medical College of Alabama, Birmingham 5, Ala *Assistant Professor of Physiology and Pharmacology* (3, 1946)
- Hitchings, George H**, Ph D 50 Primrose Ave, Tuckahoe, N Y *Chief Biochemist, Wellcome Research Laboratories* (2, 1942)
- Hjort, Axel M**, M D, Ph D P O Box 281, 14 Fern Way, Scarsdale, N Y *Adjunct Physician, Grasslands Hospital, Valhalla, N Y* (2, 1925)
- Hogland, Hudson**, M S, Ph D, Sc D (hon) 222 Maple Ave, Shrewsbury, Mass *Executive Director, Worcester Foundation for Experimental Biology, Neurophysiologist, Worcester State Hospital, and Research Professor of Physiology, Tufts College Medical School* (1, 1932)
- Hobby, Gladys L**, A B, M A, Ph D 11 Bartlett St, Brooklyn 6, N Y *Research Bacteriologist in Charge of Biological Control, Chas Pfizer & Co* (6, 1946)
- Hober, Rudolf** University of Pennsylvania Medical School, Philadelphia *Visiting Professor of Physiology* (1, 1936)
- Hodes, Robert**, Ph D Johnson Foundation, Uni-

- versity of Pennsylvania, Philadelphia *Associate in Biophysics, Professor of Physical Medicine* (1, 1941)
- Hodge, Harold Carpenter, Ph D University of Rochester School of Medicine and Dentistry, Rochester, N Y *Professor of Pharmacology and Toxicology* (2, 1937, 3, 1948)
- Hoefler, Paul F A, Ph D, M D Neurological Institute of New York, 710 W 168th St, New York 32, N Y *Associate Professor of Neurology* (1, 1945)
- Hoff, Ebbe Curtis, M A, Ph D Medical College of Virginia, Richmond 19 *Associate Professor* (1, 1933)
- Hoff, Hebbel E, M A, Ph D McGill University, Montreal, Quebec, Canada *Professor of Physiology* (1, 1933)
- Hoffman, William Samuel, Ph D, M D Hektoen Institute for Medical Research, Chicago, Ill *Director of Biochemistry, Director of Biochemistry, Cook County Hospital* (2, 1935)
- Hofmann, Klaus, Ph D Department of Chemistry, University of Pittsburgh, Pittsburgh, Penn *Research Professor* (2, 1947)
- Hogan, Albert G, A M, Ph D 105 Schweitzer Hall, Columbia, Mo *Professor of Animal Nutrition, University of Missouri* (2, 1916, 5, 1933)
- Hogness, Thorfin R, Ch E, Ph D University of Chicago, Chicago, Ill *Director, Inst of Radiobiology and Biophysics* (2, 1941)
- Holck, Harald G O, Ph D College of Pharmacy, University of Nebraska, Lincoln *Associate Professor of Pharmacology* (1, 1935, 3, 1938)
- Hollaender, Alexander, M A, Ph D Oak Ridge National Laboratory, Oak Ridge, Tenn (1, 1947)
- Hollander, Franklin, Ph D Mount Sinai Hospital, Fifth Ave and 100th St, New York City *Associate in Physiology, Head, Gastro-Enterology Research Laboratory* (1, 1942, 2, 1932)
- Holm, August, Sc D E R Squibb & Sons, New Brunswick, N J *Research Associate* (6, 1946)
- Holman, Ralph Theodore, Ph D A and M College of Texas, College Station, Texas *Associate Professor of Biochemistry and Nutrition* (2, 1948)
- Holman, Russell Lowell, M D Louisiana State University School of Medicine, New Orleans, La *Professor of Pathology* (4, 1940)
- Holmes, Arthur Dunham, Ph D University of Massachusetts, Amherst *Research Professor of Chemistry* (2, 1931, 5, 1933)
- Holmes, Joseph H, M D, D Med Sc University of Colorado School of Medicine, 4200 E Ninth Ave, Denver 7 *Assistant Professor of Physiology* (1, 1947)
- Holmes, Julia O, M S, Ph D University of Massachusetts, Amherst *Research Asst of Chemistry Dept* (2, 1942, 5, 1936)
- Holt, Joseph Paynter, M S, M D, Ph D Standard Oil Co, Room 2400, 30 Rockefeller Plaza, New York 20, N Y *Director of Medical Research* (1, 1942)
- Holt, L Emmett, Jr, M D 477 First Ave, New York 16, N Y *Professor of Pediatrics, New York University College of Medicine* (2, 1930, 5, 1946)
- Hoobler, Icie Macy, M S, Ph D, Sc D 660 Frederick St, Detroit, Mich *Director, Research Laboratory Children's Fund of Michigan* (2, 1925, 5, 1933)
- Hooker, Davenport, M A, Ph D University of Pittsburgh School of Medicine, Pittsburgh, Pa *Professor of Anatomy* (1, 1920)
- Hooker, Sanford B, A M, M D 80 E Concord St, Boston, Mass *Member, Evans Memorial* (6, 1918)
- Hoover, Sam R, M A, Ph D 7815 Linden Rd, Philadelphia 18, Pa *Sensor Chemist Eastern Regional Research Laboratory, U S Department of Agriculture* (2, 1946)
- Hoppert, C A, Ph D Michigan State College, Box 626, East Lansing *Professor of Biological Chemistry* (5, 1935)
- Hopps, Howard C, M D Department of Pathology, University of Oklahoma School of Medicine, 801 E 13th St, Oklahoma City, Okla *Professor of Pathology and Chairman of Dept* (4, 1947, 6, 1946)
- Horecker, Bernard L, Ph D National Institutes of Health, Bethesda, Md *Bichemist* (2, 1947)
- Horowitz, Norman H, Ph D California Institute of Technology, Pasadena, Calif *Associate Professor of Biology* (2, 1946)
- Horsfall, Frank L, Jr, M D, C M Rockefeller Institute, 66th St and York Ave, New York City *Member, Physician to the Hospital of the Rockefeller Inst for Medical Research* (6, 1937)
- Horton, Richard G, Ph D Army Chemical Center, Medical Division, Edgewood, Md *Acting Chief, Toxicology Section* (1, 1948)
- Horvath, Steven M, M A, Ph D Dept of Physical Medicine, Hospital of the Univ of Pennsylvania, Philadelphia, Pa (1, 1943)
- Horwitt, M K, Ph D Biochemical Research Laboratory, Elgin State Hospital, Elgin, Ill *Director, Assistant Professor, Biological Chemistry, University of Illinois School of Medicine* (2, 1941)
- Hotchkiss, Rollin D, Ph D The Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Associate* (2, 1941)
- Houck, C Riley, M A, Ph D University of Tennessee Medical School, Memphis 3 *Assistant Professor in Physiology* (1, 1947)
- Hove, Edwin L, M S, Ph D Alabama Polytechnic Institute, Laboratory of Animal Nutrition, Auburn, Ala *Research Biochemist* (5, 1946)

- Howard, Evelyn, A M , Ph D Johns Hopkins School of Medicine, Baltimore, Md *Instructor in Physiology* (1, 1933)
- Howard, John Eager, A B , M D Johns Hopkins Hospital, Baltimore 5, Md *Assistant Professor of Medicine* (1, 1946)
- Howard, Marion E , M D New Haven Hospital, New Haven, Conn *Associate Clinical Professor of Medicine, Yale School of Medicine, Associate Physician, New Haven Hospital and New Haven Dispensary* (4, 1939, 6, 1937)
- Howe, Paul E , A M , Ph D U S Dept of Agriculture, Bureau of Animal Ind , Beltsville, Md *Nutrition Consultant* (1, 1913, 2, 1909, 5, 1933)
- Howe, Percy R , M D , D D S Forsyth Dental Infirmary for Children, Boston, Mass *Director Forsyth Dental Infirmary, Professor Dental Sciences, Instructor in Pathology* (5, 1935)
- Howell, Katherine M , M D 6830 S Merrill Ave , Chicago, Ill *Head of Departments of Bacteriology and Serology* (6, 1940)
- Howell, Stacey F , Ph D V D Research Laboratory, U S Marine Hospital, Stapleton, Staten Island, N Y *Chemist, U S Public Health Service* (2, 1940)
- Hubbard, Roger Sanford, A M , Ph D University of Buffalo Sch of Med , Buffalo, N Y *Prof of Pharmacology* (1, 1922, 2, 1920)
- Hubbell, Rebecca B , M S , Ph D Connecticut Agricultural Experiment Station, New Haven *Assistant Biochemist* (2, 1937, 5, 1935)
- Hudack, Stephen Sylvester, M D 180 Fort Washington Ave , New York, N Y *Associate Professor of Orthopedic Surgery, Columbia Univ* (4, 1933)
- Huddleston, Ora Leonard, M D , Ph D Los Angeles County General Hospital, 1900 N State St , Los Angeles 33, Calif (1, 1936)
- Hueper, Wilhelm C , M D National Cancer Institute, Bethesda, Md *Chief, Environmental Cancer Section* (4, 1940)
- Huffman, C F , M S , Ph D Michigan State College, East Lansing *Research Professor and Professor in Dairy Husbandry* (5, 1937)
- Huffman, Max N , B A , Ph D Southwestern Medical College, 2211 Oak Lawn, Dallas, Texas *Research Professor of Biochemistry* (2, 1947)
- Huggins, Charles Brenton, M D University of Chicago, Chicago, Ill *Professor of Surgery* (1, 1932)
- Hughes, Hettie B , M S , Ph D The Christ Hospital, Cincinnati 19, Ohio *Research Associate* (2, 1946)
- Hughes, Joseph, M D 111 N 49th St , Philadelphia, Pa *Assistant Professor of Experimental Neurology, Graduate School of Medicine, University of Pennsylvania, Director of Laboratory, Pennsylvania Hospital for Mental Diseases* (1, 1936)
- Hughes, Josiah Simpson, M A , M S , Ph D Kansas State College, Manhattan *Professor of Chemistry* (2, 1931, 5, 1939)
- Hughes, Thomas P , A M , Ph D Caixa Postal 89, Rio de Janeiro, Brazil *Member of Staff, International Health Division* (6, 1934)
- Hulpieu, Harold R , M A , Ph D Indiana University School of Medicine, Indianapolis *Professor of Pharmacology* (3, 1939)
- Hunscher, Helen A , Ph D Western Reserve University, 2023 Adelbert Rd , Cleveland 6, O *Head of Department of Home Economics* (5, 1934)
- Hunter, Andrew, C B C , M B , F R S C Hospital for Sick Children, Toronto, Canada *Consulting Biochemist, Emeritus Professor of Pathological Chemistry, Univ of Toronto* (2, 1908)
- Hunter, Francis Edmund, Jr , Ph D Pharmacology Department, Washington University Medical School, St Louis 10, Mo *Assistant Professor of Pharmacology* (2, 1946)
- Hunter, George, M A , D Sc , F R S C University of Alberta, Edmonton, Canada *Professor of Biochemistry* (2, 1924)
- Hunter, Jesse E , M S , Ph D Allied Mills, Inc , 7500 S Adams St , Peoria, Ill *Director of Research* (5, 1936)
- Hunter, John, Ph D Univ of Michigan, Ann Arbor, Mich *Instructor in Physiology* (1, 1918)
- Hussey, Raymond, M D Medical Science Center of Wayne University, 1547 Penobscot Building, Detroit 26, Mich *Director, Institute for Occupational Health Research, Dean and Professor of Occupational Health, School of Occupational Health* (1, 1927)
- Hutchens, John O , Ph D Department of Physiology, University of Chicago, Chicago 37, Ill *Associate Professor of Physiology and Chairman of the Department, Director of the Toxicity Laboratory* (1, 1947)
- Hyman, Chester, Ph D Univ of Southern California Sch of Med , Los Angeles, Calif *Asst Professor of Physiology* (1, 1948)
- Ingalls, Mabel S , Ph D Salisbury Mills, Orange County, N Y (6, 1940)
- Ingle, Dwight J , M S , Ph D The Upjohn Co , Research Department, Kalamazoo, Mich *Upjohn Research Fellow* (1, 1939)
- Ingraham, Raymond Clifford, Ph D College of Medicine, University of Illinois, 1853 W Polk St , Chicago *Assistant Professor in Physiology* (1, 1938)
- Ingram, W R , Ph D College of Medicine, The State University of Iowa, Iowa City *Professor and Head of the Department of Anatomy* (1, 1936)

- Irish, Don D., Ph D The Dow Chemical Co, Midland, Mich *Director, Biochemical Research Laboratory* (3, 1948)
- Irvine, J Logan, Ph D Johns Hopkins University School of Medicine, 710 N Washington St, Baltimore, Md *Assistant Professor of Physiological Chemistry* (2, 1942)
- Irving, George Washington, Jr, M A, Ph D U S Dept of Agriculture, Bur of Ag and Ind Chem, Washington, D C *Assistant Chief* (2, 1946)
- Irving, Laurence, A M, Ph D Swarthmore College, Swarthmore, Pa *Lt Col, A C Professor of Experimental Biology* (1, 1927)
- Irwin, M R, Ph D Department of Genetics, University of Wisconsin, Madison *Professor of Genetics* (6, 1936)
- Isaacs, Raphael, M D 104 S Michigan Ave, Suite 630, Chicago 3, Ill *Attending Physician, Department of Hematology, Michael Reese Hospital* (4, 1928)
- Isenberger, R M, M A, M D University of Kansas School of Medicine, Kansas City *Professor of Pharmacology* (3, 1937)
- Iwamoto, Harry K, Ph D Univ of Maryland School of Medicine, Baltimore, Md *Asst Professor of Pharmacology* (3, 1948)
- Ivy, A C, Ph D, M D, D Sc University of Illinois, Chicago Professional Colleges, Chicago, Ill *Vice-President, Distinguished Professor of Physiology* (1, 1919, 5, 1933)
- Izquierdo, J Joaquin M D National School of Medicine, Mexico City *Professor of Physiology in the National School of Medicine and the Escuela Medico Militar of Mexico* (1, 1928)
- Jackson, Dennis Emerson, A M, Ph D M D University of Cincinnati Medical School, Eden and Bethesda Aves, Cincinnati, O *Professor of Pharmacology* (1, 1910, 3R, 1912)
- Jackson, Eugene L, Ph D 12 S 12th St, Richmond 19, Va *Medical Director, A H Robins Co* (3, 1942)
- Jackson, Richard W, Ph D Northern Regional Research Laboratory, U S Department of Agriculture, 825 N University St, Peoria 5, Ill *Head of Fermentation Division* (2, 1930, 5, 1933)
- Jacobs Merkel Henry, Ph D University of Pennsylvania, Philadelphia *Professor of General Physiology, Member of the National Academy of Sciences* (1, 1919)
- Jacobs, Walter A, A M, Ph D Rockefeller Institute, 66th St and York Ave, New York City *Member, Member, National Academy of Sciences* (2, 1908, 3, 1913)
- Jacobson, Edmund, Ph D, M D Laboratory for Clinical Physiology, 55 E Washington St, Chicago, Ill (1, 1929)
- Jaffe, Henry L, M D Hospital for Joint Diseases, 1919 Madison Ave, New York City *Director of Laboratories* (4, 1925)
- Jahn, Theodore Louis, Ph D University of California, Los Angeles, Calif *Dept of Zoology* (1, 1944)
- Jailer, Joseph W, Ph D Columbia Univ Coll of Phys and Surg, New York City *Instructor in Medicine* (1, 1948)
- Jamieson, Walter A, Sc D (hon) Eli Lilly & Company, Indianapolis, Ind *Director, Biological Division* (6, 1927)
- Jandorf, Bernard J, A M, Ph D, Medical Division, Army Chemical Center, Md *Research Biochemist, Biochemistry Section, Lecturer in Preventive Medicine, Johns Hopkins University School of Medicine* (2, 1946)
- James, Ralph G, Ph D State Univ of Iowa Coll of Med, Iowa City, Iowa *Asst Professor of Anatomy* (1, 1948)
- Jansen, Eugene F, B A Enzyme Research Laboratory, Western Regional Research Laboratory, Albany 6, Calif *Senior Chemist, Bureau of Agricultural and Industrial Chemistry, U S Department of Agriculture* (2, 1947)
- Jacques, L B, M A, Ph D Univ of Saskatchewan, Saskatoon, Sask, Canada *Professor of Physiology* (1, 1943)
- Jasper, Herbert H, M A, Ph D, D és Sci Montreal Neurological Institute, 3801 University St, Montreal, Que, Canada *Lecturer in Neuroelectrography and Director of Department of Electrophysiology* (1, 1940)
- Jears, P C, M D State University of Iowa, Iowa City *Professor of Pediatrics* (5, 1937)
- Jensen, Hans F Ph D Medical Research Field Laboratory, Fort Knox, Kentucky *Chief Biochemist* (2, 1929)
- Jochim, Kenneth E, Ph D Dept of Physiology, Univ of Kansas, Lawrence (1, 1942)
- Johlin J M, Ph D D Sc Vanderbilt University School of Medicine, Nashville Tenn *Associate Professor of Biochemistry* (2, 1928)
- Johnson, B Connor, M A, Ph D, Division of Animal Nutrition, University of Illinois, Urbana, Ill *Assoc Professor* (2, 5, 1947)
- Johnson, Frank H, A M, Ph D Princeton University, Princeton N J *Assistant Professor Dept of Biology* (1, 1942)
- Johnson, Joseph L, Ph D, M D School of Medicine, Howard University, Washington, D C *Professor and Head of the Department of Physiology* (1, 1934)
- Johnson, J Raymond, Ph D University of Ottawa, Faculty of Medicine, Ottawa, Ontario, Canada (1, 1938)
- Johnson, Marvin J, M S, Ph D University of Wisconsin, Madison *Professor of Biochemistry* (2, 1941)

- Johnson, Robert E**, M D, Ph D Army Medical Nutrition Laboratory, 1849 W Pershing Rd, Chicago 9, Ill *Director* (1, 1944, 2, 1939, 5, 1946)
- Johnson, S R**, M S, Ph D Missouri Farmers Association Milling Co, Springfield, Mo *Director of Nutrition* (5, 1947)
- Johnson, Victor**, Ph D, M D Mayo Foundation, Rochester, Minn (1, 1933)
- Johnston, Charles G**, M S, M D Wayne University College of Medicine, Detroit, Mich *Professor of Surgery* (1, 1933)
- Johnston, Frances A**, Ph D Cornell Univ, Ithaca, N Y *Asst Professor of Foods and Nutrition* (5, 1948)
- Johnston, Margaret W**, Ph D B 452, University Hospital, Ann Arbor, Mich *Research Associate in Internal Medicine* (2, 1930, 5, 1938)
- Jolliffe, Norman**, M D 39 E 75th St, New York, N Y (1, 1932)
- Jones, D Breese**, Ph D Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U S Department of Agriculture, Beltsville, Md *Protein Chemist* (2, 1920, 5, 1935)
- Jones, James H**, M S, Ph D School of Veterinary Medicine, University of Pennsylvania, Philadelphia *Professor of Physiological Chemistry* (2, 1928, 5, 1933)
- Jones, Kenneth K**, M S, Ph D Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill *Associate Professor of Physiology and Pharmacology* (1, 1936)
- Joseph, Norman R**, Ph D University of Illinois, 1853 West Polk Street, Chicago 12, Ill *Assistant Professor of Chemistry* (2, 1917)
- Joslin, Elliott P**, M A, M D New England Deaconess Hospital, 81 Bay State Rd, Boston, Mass *Director, George F Baker Clinic* (5, 1933)
- Jukes, Thomas Hughes**, Ph D Lederle Laboratories, Pearl River, N Y *Head, Department of Nutrition and Physiology Research* (2, 1935, 5, 1938)
- Jung, Frederic Theodore**, Ph D, M D American Medical Association, Chicago, Ill *Assistant Secretary* (1, 1930)
- Jungeblut, Claus W**, M D College of Physicians and Surgeons, 630 W 168th St, New York City *Professor of Bacteriology, Columbia University* (4, 1929, 6, 1926)
- Kabat, Elvin A**, A M, Ph D Columbia University Coll of Phys and Surg, 710 W 168th St, New York City *Assoc Prof of Bacteriology* (2, 1940, 6, 1943)
- Kabat, Herman**, Ph D, M D 2633 16th St, N W Washington, D C *Consultant in Neurology, Health Department, District of Columbia* (1, 1941)
- Kahn, Reuben L**, Sc D, LL D University of Michigan Hospital, Ann Arbor *Associate Professor of Serology of Syphilis and Chief of Serology Laboratory* (4, 1934, 6, 1919)
- Kalckar, Herman M**, M D, Ph D Institute for Medical Physiology, University of Copenhagen, 25 Juliane Varies Vej Copenhagen, Denmark *Associate Professor* (2, 1912)
- Kalnitsky, George**, Ph D State Univ of Iowa, Iowa City, Iowa *Asst Professor of Biochemistry* (2, 1948)
- Kamen, Martin D**, Ph D Washington University Medical School, 510 S Kingshighway, St Louis 10, Mo *Associate Professor of Chemistry* (2, 1916)
- Kamm, Oliver**, Ph D 365 Lakeshore Drive, Detroit, Mich *Scientific Director, Research Laboratory, Parke, Davis & Co* (2, 1928)
- Karel, Leonard**, Ph D Research Grants Division, National Institute of Health, Bethesda 14, Md *Ex Asst Antibiotics and Pharmacology Study Sections, Univ of Md Sch of Med, Lecturer in Pharmacology* (3, 1917)
- Karpovich, Peter V**, M D, M P L Springfield College, Springfield, Mass *Professor of Physiology* (1, 1912)
- Karshan, Maxwell**, Ph D Department of Biological Chemistry, Columbia University, 630 W 168th St, New York City *Associate Professor of Biochemistry* (2, 1939)
- Karsner, Howard T**, M D Western Reserve University, 2085 Adelbert Rd, Cleveland, O *Professor of Pathology, Director of the Institute of Pathology* (4, 1913, 6, 1925)
- Katz, Louis Nelson**, A M, M D 2900 Ellis Ave, Chicago, Ill *Director of Cardiovascular Research, Michael Reese Hospital, Professorial Lecturer in Physiology, University of Chicago* (1, 1921)
- Katzman, Philip A**, Ph D St Louis University School of Medicine, 1402 S Grand Blvd, St Louis 1, Mo *Associate Professor of Biochemistry* (2, 1935)
- Kaulbersz, Jerzy**, Ph D, M D Collegium Medica, Grzegorzeczka 16, Cracow, Poland *Professor of Physiology* (1, 1944)
- Kaunitz, Hans**, M D College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, N Y *Research Associate in Pathology* (4, 1917)
- Kay, Herbert Davenport**, Ph D, D Sc, F R S National Institute for Research in Dairying, Shinfield, near Reading, England *Director, Research Professor of Biochemistry, University of Reading* (2, 1930)
- Kazal, Louis Anthony**, Ph D Medical Research Division, Sharp & Dohme, Inc, Glenolden, Penn *Research Biochemist* (2, 1947)
- Keeton, Robert W**, M S, M D 1817 W Polk St, Chicago *Professor of Medicine, Univ of Illinois Coll of Medicine* (1, 1916, 3, 1924)
- Kehoe, Robert A**, M D Kettering Laboratory

- of Applied Physiology, College of Medicine, University of Cincinnati, Eden Ave, Cincinnati, O *Research Professor of Physiology* (1, 1940)
- Keith, Norman M, M D Mayo Clinic, Rochester, Minn *Consulting Physician, Division of Medicine, Mayo Clinic, Professor of Medicine, Mayo Foundation, University of Minnesota* (1, 1920, 3, 1932, 4, 1924)
- Keith, T B, Ph D Dept of Animal Husbandry, University of Idaho, Moscow, Idaho (5, 1941)
- Kellaway, Peter E, M A, Ph D Baylor University, College of Medicine, Houston, Texas (1, 1947)
- Keller, Allen D, Ph D Field Research Lab, Fort Knox, Kentucky *Medical Dept* (1, 1931)
- Kelser, Raymond A, D V M, Ph D 130 Valley Rd, Ardmore, Pa *Professor of Bacteriology and Dean of Faculty School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa* (4, 1932)
- Kelsey, F Ellis B S, Ph D University of Chicago, Chicago, Ill *Research Associate (Instructor) in Pharmacology* (3, 1941)
- Kelsey, Frances Kathleen O, M S, Ph D University of Chicago, Chicago, Ill *Assoc Professor of Pharmacology* (3, 1941)
- Kemmerer, A R, Ph D University of Arizona, Tucson, Arizona *Head, Dept of Human Nutrition* (5, 1946)
- Kempner, Walter, M D Duke University School of Medicine, Durham, N C *Assistant Professor of Medicine* (1, 1940)
- Kendall, Edward C, M S, Ph D, D Sc 627 Eighth Ave, S W, Rochester, Minn *Professor of Biochemistry, Mayo Foundation, University of Minnesota* (1, 1916, 2, 1913, 4, prior to 1920)
- Kendall, Forrest E, Ph D 240-06-53rd Ave Douglaston, Long Island, N Y *Assistant Professor of Biochemistry, Research Service, Columbia Division, Goldwater Memorial Hospital, Welfare Island, N Y* (6, 1943)
- Kennard, Margaret A, M D University of Oregon, Medical School, Dept of Surgery, Portland, Ore *Assoc Professor of Experimental Surgery* (1, 1934)
- Kennedy, Cornelia, Ph D 2700 W Robbins St, Minneapolis, Minn (2, 1924, 5, 1945)
- Kennedy, Robert P, M D Knollwood Drive, R D 1, Rochester, N Y (4, 1929)
- Kent, John F, A M Army Medical Center, Washington 12, D C *Scientific Director, Department of Serology, Army Medical Department Research and Graduate School* (6, 1947)
- Kenton, Harold B, Ph D New England Deaconess Hospital, Boston, Mass *Bacteriologist and Director of the Blood Bank* (6, 1934)
- Kenyon, Allan T, M D University of Chicago, Division of Biological Sciences, 950 E 59th St, Chicago, Ill *Assistant Professor of Medicine* 3, 1940)
- Keresztesy, John C, Ph D National Institute of Health, Bethesda 14, Md *Scientist Officer, Division of Physiology* (2, 1941, 5, 1945)
- Kerr, Stanley E, Ph D American University of Beirut, Beirut, Lebanon, Syria *Professor of Biological Chemistry* (2, 1937)
- Kerr, Wm J, M D University of California Hospital, Third and Parnassus Aves, San Francisco *Professor of Medicine, University of California, Physician-in-Chief, University of California Hospital* (3, 1930)
- Kesten, Homer D, M D College of Physicians and Surgeons, Columbia University, New York City *Associate Professor of Pathology* (4, 1931)
- Kety, Seymour S, M D Dept of Pharmacology, Medical School, University of Pennsylvania, Philadelphia 4 *Associate in Pharmacology, Medical School, Assistant Visiting Physician in Medicine, Philadelphia General Hospital* (3, 1945)
- Keys, Ancel, M A, Ph D, D Phil Stadium South Tower, University of Minnesota, Minneapolis *Professor and Director of Laboratory of Physiological Hygiene* (1, 1939, 2, 1936)
- Kidd, John G, M D Cornell University Medical College, 1300 York Ave, New York City *Professor of Pathology, Pathologist, New York Hospital* (4, 1938, 6, 1937)
- Kies, Marian W, Ph D 2827 Otis St N E, Washington, D C (2, 1948)
- Kik, M C, Ph D College of Agriculture, University of Arkansas, Fayetteville *Associate Professor of Agricultural Chemistry* (5, 1942)
- Kilborn, Leslie G, M A, M D, Ph D 47 Warren Road, Toronto, Ontario, Canada At present West China Union University, Chengtu, Sze, China (1, 1928)
- Killian, John Allen, A M, Ph D Killian Research Laboratories, Inc, 49 W 45th St, New York City (2, 1921)
- Kinard, F W, M S, Ph D, M D Medical College of the State of South Carolina, Charleston 16 *Associate Professor of Physiology* (1, 1947)
- King, Barry G, M A, Ph D Medical Service, Safety Regulations, Civil Aeronautics Administration, Department of Commerce, Washington, D C *Chief, Aeromedical Design and Material Division* (1, 1938)
- King, Charles Edwin, Ph D Vanderbilt University, Nashville, Tenn *Associate Professor of Physiology* (1, 1916)
- King, Charles Glen, Ph D Nutrition Foundation, Inc, Chrysler Building, New York City *Scientific Director, Professor of Chemistry, Columbia University* (2, 1931, 5, 1933)
- King, Joseph T, M D, Ph D 314 Millard Hall,

- University of Minnesota Medical School, Minneapolis *Associate Professor of Physiology* (1, 1931)
- Kirch, Ernst R, Ph D Univ of Illinois College of Pharmacy, 808 S Wood St, Chicago, Ill *Assoc Professor of Chemistry* (2, 1918)
- Kirchhof, Anton C, M S, M D University of Oregon Medical School, Portland 1, Ore *Research Associate, Dept of Pharmacology* (3, 1947)
- Kirk, Paul L, Ph D University of California, Berkeley *Professor of Biochemistry* (2, 1933)
- Kirkbride, Mary B, Sc D 314 State St, Albany 6, N Y (6, 1921)
- Kirschbaum, Arthur, M D, Ph D Univ of Minnesota Medical School, Minneapolis, Minn *Assoc Professor of Anatomy* (4, 1948)
- Kisch, Bruno, M D Yeshiva University, New York City *Professor of Biochemistry* (1, 1943)
- Kleiber, M, D Sc University of California, Davis *Professor of Animal Husbandry* (1, 1913, 5, 1933)
- Klein, J Raymond, Ph D Brookhaven National Lab, Upton, L I, N Y *Sr Scientist* (2, 1941)
- Kleiner, Israel Simon, Ph D New York Medical College and Flower Hospital, New York 29, N Y *Professor of Biochemistry, Director Dept of Physiology and Biochemistry* (1, 1911, 2, 1912, 3R, 1912, 5, 1933)
- Kleitman, Nathaniel, A M, Ph D University of Chicago, Chicago, Ill *Associate Professor of Physiology* (1, 1923)
- Klemperer, Friedrich Wilhelm, M D Trudeau Foundation, Trudeau, N Y *Head of Department of Biochemistry* (2, 1941)
- Kletzien, Seymour W, M S, Ph D, 330 S Ninth St, Philadelphia, Pa *Nutrition Research Clinic, Philadelphia Lying-In & Pennsylvania Hospitals Biochemist* (5, 1933)
- Kline, O L, Ph D Federal Security Agency, Food and Drug Administration, Washington, D C *Biochemist* (5, 1936)
- Kline, Raymond F, B S, M S Physiological Lab Univ of Virginia Med School, Charlottesville, Va *Instructor in Physiology* (1, 1946)
- Klotz, Irving M, Ph D Department of Chemistry, Northwestern University, Evanston, Ill *Associate Professor of Chemistry* (2, 1947)
- Kluver, Heinrich, Ph D University of Chicago, Chicago, Ill *Professor of Experimental Psychology* (1, 1935)
- Knight, C Arthur, Ph D The Virus Laboratory, University of California, Berkeley 4, Calif *Assoc Professor* (2, 1946)
- Knoefel, Peter K, M A, M D University of Louisville, 101 W Chestnut St, Louisville, Ky *Professor of Pharmacology* (3, 1934)
- Knox, Eugene W, M D 3026 S California Ave, Chicago, Ill *Research Associate, Rheumatic Fever Research Institute, Northwestern Univ Medical School* (2, 1948)
- Knowlton, G Clinton, Ph D Room 101, Physiology Bldg, Emory University, Ga (1, 1938)
- Knudson, Arthur, Ph D Albany Medical College, New Scotland Ave, Albany, N Y *Professor of Biochemistry and Associate Dean* (2, 1919, 5, 1936)
- Knutti, Ralph Eddy, M D Children's Hospital, Los Angeles, Calif *Director of Laboratories, Assistant Professor of Pathology, University of Southern California* (1, 1933)
- Kober, Philip A, B S Sherman Laboratories, Detroit, Mich *Director of Research* (2, 1912)
- Kobrak, Heinrich G, Ph D Univ of Chicago, Chicago, Ill *Asst Professor of Surgery* (1, 1918)
- Kochakian, Charles D, Ph D University of Rochester School of Medicine and Dentistry, Rochester, N Y *Associate Professor of Physiology (Endocrinology)* (1, 1912, 2, 1918)
- Kocher, Rudolph Alfred, M D Box 936, Carmel, Calif *Director, Velie Metabolic Clinic* (2, 1915)
- Koehler, Alfred E, M D, Ph D 317 W Pueblo St, Santa Barbara, Calif *Physician, Sansum Clinic, Santa Barbara Cottage Hospital* (2, 1924)
- Koehne, Martha, Ph D 285 15th Ave, Apt 22, Columbus, Ohio *Nutritionist, Ohio State Dept of Health* (5, 1933)
- Koelle, George B, B Sc, Ph D Wilmer Institute, Johns Hopkins Hospital, Baltimore, Md *Chalfont Fellow in Ophthalmology* (3, 1947)
- Koeppf, George F, M D 537 Delaware Ave, Buffalo 2, N Y *Associate in Physiology, University of Buffalo* (1, 1942)
- Koerber, Walter L, Ph D E R Squibb & Sons, New Brunswick, N J *Assistant Department Head* (6, 1943)
- Kohlstaedt, Kenneth G, M D Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis 7, Ind *Director* (1, 1947)
- Kohn, Henry I, Ph D Oak Ridge Nat'l Lab, Oak Ridge, Tenn *Surgeon, USPHS* (1, 1940)
- Kolmer, John A, M S, M D, D PH, Sc D, LL D, L H D 1 Montgomery Ave, Bala-Cynwyd, Pa *Professor of Medicine, Temple University, Director, Research Institute of Cutaneous Medicine* (6, 1913)
- Komarov, Simon A, M S, M D, Ph D S S Fels Fund, Med Research Laboratory, 255 S 17th St, Philadelphia, Pa *Director of Dept of Biochemistry* (1, 1933)
- Kopeloff, Nicholas, Ph D New York State



- Psychiatric Institute, 722 W 168th St, New York City *Principal Research Bacteriologist, New York State Psychiatric Institute and Hospital* (6, 1937)
- Koppányi, Theodore, Ph D Georgetown University, Washington, D C *Professor of Pharmacology* (1, 1924, 3, 1935)
- Koprowski, Hillary, M D Lederle Laboratories Division, American Cyanamid Co, Pearl River, N Y *Asst Director, Viral and Rickettsial Research* (6, 1946)
- Korr, Irwin M, M A, Ph D Kirksville College of Osteopathy & Surgery, Kirksville, Mo *Professor of Physiology* (1, 1939)
- Kottke, Frederic J, M S, Ph D, M B, M D University of Minnesota, Minneapolis *Baruch Fellow in Physical Medicine* (1, 1947)
- Kozelka, Frank L, Ph D Dept of Pharmacology and Toxicology, University of Wisconsin, Madison *Assoc Professor of Toxicology* (3, 1939)
- Krahl, Maurice E, Ph D Washington Univ School of Medicine, St Louis 10, Mo *Assistant Professor of Pharmacology* (2, 1939)
- Krakower, Cecil Alexander, M D University of Illinois College of Medicine, 1853 West Polk St, Chicago *Associate Professor of Pathology* (4, 1945)
- Kramer, Benjamin, A M, M D 60 Plaza St, Brooklyn, N Y *Pediatrician in Chief, Brooklyn Jewish Hospital, Professor of Clinical Pediatrics, Long Island College Medical School* (1, 1915, 2, 1914)
- Kramer, Martha, Ph D Kansas State College, Manhattan *Assistant Dean, School of Home Economics* (5, 1933)
- Kramer, S D, M D, Ph D 2635 36th Ave, St Petersburg, Fla *Virologist* (6, 1944)
- Krampitz, Lester O, Ph D Western Reserve Univ, Cleveland, Ohio *Head, Dept of Microbiology* (2, 1946)
- Krantz, John C, Jr, M S, Ph D University of Maryland Medical School, Baltimore *Professor of Pharmacology* (3, 1937)
- Krauss, William E, Ph D Ohio Agricultural Experiment Station, Wooster *Associate Director* (2, 1932, 5, 1933)
- Kraybill, Henry R, M S, Ph D 5720 Woodlawn Ave, Chicago 37, Ill *Professorial Lecturer, Department of Biochemistry, University of Chicago, Director, Department of Scientific Research, American Meat Institute Foundation* (2, 1942)
- Krayer, Otto, M D Harvard Medical School, 25 Shattuck St, Boston, Mass *Associate Professor of Comparative Pharmacology* (3, 1938)
- Kreezer, George L, Ph D Washington Univ, St Louis, Mo *Assoc Professor of Psychology* (1, 1948)
- Krehl, Willard A, Ph D Yale University, 333 Cedar Street, New Haven, Conn *Assistant Professor of Nutrition* (2, 1947)
- Krichesky, Boris, Ph D Univ of California, Los Angeles, Calif *Assoc Professor and Chairman, Dept of Zoology* (1, 1948)
- Krop, Stephen, M S, Ph D Medical Division, Army Chemical Center, Edgewood, Md *Chief, Pharmacology Section* (3, 1944)
- Krueger, Albert Paul, M D University of California, Berkeley *Professor of Bacteriology, and Lecturer in Medicine Chairman, Department of Bacteriology, Director, Office of Naval Research Task V* (4, 1930, 6, 1937)
- Krueger, Hugo M, Ph D Department of Zoology, Oregon State College, Corvallis, Oregon *Prof of Physiology* (1, 1931, 3, 1935)
- Krumbhaar, Edward B, M D, Ph D University of Pennsylvania Medical School, Philadelphia *Professor of Pathology* (1R, 1914, 4, prior to 1920)
- Kruse, Harry Dayton, M D, Sc D Milbank Memorial Fund, 40 Wall St, New York City (2, 1933)
- Kruse, Theophile K, A M, Ph D University of Pittsburgh Medical School, Pittsburgh, Pa *Professor of Physiology and Pharmacology* (1, 1919, 3, 1920)
- Kubicek, William G, Ph D Department of Physiology, University of Minnesota, Minneapolis *Assistant Professor* (1, 1947)
- Kubie, Lawrence S, M D Yale University School of Medicine, New York City *Clinical Professor of Psychiatry and Mental Hygiene* (4, 1928)
- Kuhn, Harry A, M S, Ph B 3915 Fulton St, N W, Washington, D C *Colonel, C W S, War Department, Executive Officer, C W Procurement District* (3, 1927)
- Kuhn, L Roland, Ph D AMDR GS, Army Medical Center, Washington 12, D C *Chief, Dept of Bacteriology, Army Medical Dept Research and Graduate School* (6, 1939)
- Kuizenga, Marvin H, M Sc, Ph D The Upjohn Company, Kalamazoo, Mich *Department Head, Pharmacology Endocrinology* (2, 1947)
- Kunde, Margarete M, Ph D, M D 30 N Michigan Ave, Chicago, Ill *Instructor in Medicine, Northwestern University Medical School, Clinical Assistant in Endocrinology, Cook County Hospital* (1, 1924)
- Kunitz, Moses, Ph D The Rockefeller Institute for Medical Research, Princeton, N J *Associate Member* (2, 1947)
- Kurotchkin, Timothy J, M D 156 Forest Ave, Pearl River, N Y *Bacteriologist* (6, 1946)
- Kurtz, Alton C, Ph D Department of Biochemistry, Medical School, University of Oklahoma, Oklahoma City *Associate Professor* (2, 1942)
- Kuyper, Adrian C, M S, Ph D Wayne University College of Medicine, Detroit 26, Mich *As-*

- stant Professor of Physiological Chemistry (2, 1946)
- Kydd, David M**, M D Yale University School of Medicine, New Haven, Conn Associate Professor of Medicine (5, 1934)
- Kyker, Granvil C**, Ph D Department of Biological Chemistry and Nutrition, University of North Carolina School of Medicine, Chapel Hill, N C Associate Professor (2, 1947)
- LaBelle, Annette**, B A Grasslands Hospital, Valhalla, New York (1, 1918)
- Lackey, Robert W**, M S, Ph D Department of Physiology and Pharmacology, Southwestern Medical College, Dallas 4, Texas Professor of Physiology (1, 1947)
- Lacorte, Jose G**, M D Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, S A Director of the Virus Section of the Oswaldo Cruz Institute (6, 1946)
- Lacy, G R**, M D University of Pittsburgh, Pittsburgh, Pa Professor of Bacteriology and Immunology (4, 1927)
- Lalich, Joseph J**, M D Dept of Pathology, University of Wisconsin, 426 North Charter St, Madison 6, Wis Assistant Professor of Pathology (4, 1946)
- Lamb, Alvin R**, M S, Ph D Experiment Station, Hawaiian Sugar Planters' Association, Honolulu Research Associate (2, 1923, 5, 1934)
- Lambert, Edward H**, Ph D, M D Mayo Foundation, Rochester, Minn Assistant Professor of Physiology (1, 1945)
- Lambert, Robert A**, M D Buicks Springs, Greensboro, Ala (4, 1922)
- Lambertsen, Christian J**, M D Univ of Pennsylvania, Philadelphia, Pa Asst Professor of Pharmacology, Asso in Medicine, Markle Scholar in Medical Science (3, 1918)
- Lampen, J Oliver**, Ph D Washington University School of Medicine, St Louis, Mo Asst Prof of Biological Chem (2, 1947)
- Lamport, Harold**, M D Yale University School of Medicine, New Haven, Conn Associate Professor of Physiology (1, 1943)
- Lamson, Paul Dudley**, M D Vanderbilt University Medical School, Nashville, Tenn Professor of Pharmacology (1, 1921, 3, 1915)
- Lancefield, Rebecca C**, Ph D 66th St and York Ave, New York City 21 Associate Member, Rockefeller Institute for Medical Research (6, 1933)
- Landis, Carney**, Ph D Psychiatric Institute and Hospital, Columbia University, 722 W 168th St, New York City Principal Research Psychologist and Professor of Psychology (1, 1939)
- Landis, Eugene Markley**, Ph D, M D Department of Physiology, Harvard Medical School, 25 Shattuck St, Boston, Mass George Higginson Professor of Physiology (1, 1928)
- Landowne, Milton**, M D 2650 Wisconsin Ave, N W, Washington, D C (1, 1917)
- Lands, Alonzo M**, M A, Ph D Sterling-Winthrop Research Institute, Rensselaer, N Y Head, Pharmacology Section (1, 1942, 3, 1947)
- Lange, Carl**, M D 371 Morris St, Albany, N Y Associate Bacteriologist, Divisions of Laboratories and Public Health, New York State Department of Health (6, 1938)
- Langley, Wilson D**, Ph D University of Buffalo Medical School, Buffalo, N Y Professor of Biochemistry (2, 1937)
- Langworthy, Orthello R**, M A, M D Johns Hopkins Hospital, Baltimore, Md Associate Professor of Psychiatry, Johns Hopkins University (1, 1928)
- Lardy, Henry A**, M S, Ph D Dept of Biochemistry, University of Wisconsin, Madison 6, Wis Associate Professor (2, 1916)
- Larrabee, Martin G**, Ph D Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia Associate Professor of Biophysics (1, 1910)
- Larson, Carl L**, M D Div of Infectious Diseases, National Institutes of Health, Bethesda, Md Surgeon, USPHS (6, 1948)
- Larson, Edward**, Ph D University of Miami, Miami, Fla Professor of Physiology and Pharmacology (1, 1929, 3, 1937)
- Larson, Hardy W**, A M, Ph D Metropolitan Life Insurance Co, Biochemical Laboratory, 1 Madison Ave, New York City Research Chemist (2, 1937)
- Larson, Paul S**, Ph D Medical College of Virginia, Richmond Associate Professor of Research Pharmacology (1, 1939, 3, 1947)
- Lashley, K S**, M S, Ph D, D Sc Yerkes Laboratories, Orange Park, Fla Research Professor of Neuropsychology, Harvard University, Director, Yerkes Laboratories of Primate Biology, Inc Member of the National Academy of Sciences (1, 1923)
- Laskowski, M**, Ph D Marquette University Medical School, Milwaukee 3, Wis Associate Professor of Biochemistry (2, 1944)
- Last, Jules H**, Ph D, M D Univ of Illinois College of Medicine, Chicago, Ill Asst Professor of Pharmacology (3, 1948)
- Lauffer, Max A, Jr**, M S, Ph D 307 Thaw Hall, University of Pittsburgh, Pittsburgh, Pa Research Professor (2, 1946)
- Laug, E P**, M A, Ph D Division of Pharmacology, Food and Drug Administration, 12th and C Sts SW, Washington 25, D C Senior Pharmacologist (2, 1938, 3, 1947)
- Lauson, Henry D**, Ph D, M D The Rockefeller

- Institute, 66th St & York Ave New York 21, N Y *Associate* (1, 1946)
- Lavine, T F, Ph D Lankenau Hospital Research Institute, Philadelphia, Pa *Research Chemist* (2, 1938)
- Lawrence, W Sherwood, M D 906 Hazel St, Gridley, Calif (3, 1944)
- Lawson, Hampden, M D, Ph D University of Louisville, Louisville, Ky *Professor of Physiology* (1, 1933)
- Lawton, Alfred H, M D, Ph D Veterans Administration, Bureau of Medicine and Surgery, Washington, D C (3, 1948)
- Leake, Chauncey D, M S, Ph D The University of Texas Medical Branch, Galveston *Vice-President of the University of Texas in Charge of the Medical Program* (1, 1923, 3, 1924)
- Leathem, James H, Ph D Rutgers University, New Brunswick, N J *Assistant Professor of Zoology* (1, 1945)
- Leathes, John Beresford, M A, M B, F R C S, F R S Westfield Ware Lane, Lyme Regis, Dorset, England (2, 1909)
- Lederer, Ludwig George, Ph D, M D Pennsylvania Central Airlines, National Airport, Washington, D C *Medical Director* (1, 1940)
- Lee, Milton O, M A, Ph D 2101 Constitution Ave, Washington 25, D C *Executive Secretary and Managing Editor, American Physiological Society, Federation Secretary* (1, 1927, 5, 1933)
- Leese, Chester E, M S, Ph D George Washington University School of Medicine, Washington, D C *Associate Professor of Physiology* (1, 1934)
- Lehman, Arnold J, Ph D, M D Food and Drug Administration, Washington 25, D C *Chief, Division of Pharmacology* (3, 1937)
- Lehman, Robert A, M S, Ph D New York University College of Medicine, 477 First Ave, New York City *Instructor in Therapeutics* (3, 1942)
- Lehmann, Gerhard, M D, Dr Ing Scientific Department, Hoffmann-La Roche, Nutley 10, N J *Pharmacologist* (3, 1939)
- Lehninger, Albert L, M S, Ph D University of Chicago Medical School, Chicago, Ill *Assistant Professor of Biochemistry in the Depts of Biochemistry and Surgery* (2, 1946)
- Lehr, David, M D New York Medical College, Flower and Fifth Avenue Hospitals, Fifth Ave at 105th Street, New York 29, N Y *Assistant Professor of Pharmacology* (3, 1947)
- Leichsenring, Jane M, Ph D Univ of Minnesota, University Farm, St Paul, Minn *Professor of Nutrition* (5, 1948)
- Leimdorfer, Alfred, M D Department of Psychiatry, University of Illinois College of Medicine, Chicago 12 *Associate Professor* (1, 1947)
- Lein, Allen, B A, M A, Ph D Department of Physiology, Northwestern University Medical School, Chicago 11, Ill *Physiologist* (1, 1946)
- Lenhart, Carl H, M D Lakeside Hospital, 2065 Adelbert Rd, Cleveland, O *Oliver H Payne Professor of Surgery, Western Reserve University* (1, 1921)
- Lennette, Edwin H, Ph D, M D California State Dept of Public Health, Berkeley, Calif *Director, Viral and Rickettsial Disease Laboratory* (4, 1941, 6, 1947)
- Leonard, Clifford Shattuck, M S, Ph D 87 Oxford Rd, Longmeadow, Springfield, Mass Yale University Medical School, New Haven, Conn *Technical Associate, Chemical-Biological Coordination Center, National Research Council* (3, 1927)
- Leonards, Jack Ralph, Ph D Western Reserve Univ, Cleveland, Ohio *Asst Professor of Clinical Chemistry* (2, 1948)
- Lepkovsky, Samuel, M S, Ph D University of California, Poultry Division, Berkeley *Professor* (2, 1933, 5, 1933)
- L'Esperance, Elise L, M D 2 East 61st St, New York, N Y *Director, Strong Cancer Precaution Clinic, Memorial Hospital, and New York Infirmary* (6, 1920)
- Leverson, Ruth M, Ph D Department of Home Economics, University of Nebraska, Lincoln *Associate Professor Human Nutrition Research* (5, 1942)
- Levin, Louis, Ph D College of Physicians and Surgeons, Columbia Univ, 630 W 168th St, New York 32, N Y *Assistant Professor of Anatomy* (2, 1939)
- Levine, Harold, Ph D 1037 McKinley Ave, Milwaukee, Wis *Biochemist, Research Labs, Pabst Brewing Co* (2, 1933, 5, 1933)
- Levine, Milton, M S, Ph D Inst of Experimental Medicine, College of Medical Evangelists, 312 N Boyle Ave, Los Angeles, Calif *Assoc Director, Inst of Experimental Medicine* (6, 1942)
- Levine, Philip, M A, M D, F A C P Ortho Research Foundation, Raritan, N J *Director, Biologic Division* (6, 1925)
- Levine, Rachmiel, M D, C M Michael Reese Hospital, Chicago, Ill *Acting Director, Department of Metabolic Research Professorial Lecturer in Physiology, University of Chicago* (1, 1942)
- Levine Samuel Z, M D, New York Hospital, 525 E 68th St, New York City *Professor of Pediatrics, Cornell University Medical College, Pediatrician-in-Chief, New York Hospital* (5, 1933)
- Levine, Victor Emanuel, A M, Ph D, M D Creighton University School of Medicine,

- Omaha, Nebr *Professor of Biological Chemistry and Nutrition* (2, 1936)
- Levinson, Samuel A , Ph D , M D University of Illinois College of Medicine, 808 S Wood St , Chicago *Professor of Pathology, Director Laboratories, Research & Educational Hospital* (1, 1938)
- Levison, Louis A , M D 421 Michigan St , Toledo, O *Physician to Toledo Hospital, Physician to St Vincent Hospital* (6, 1916)
- Levy, Milton, Ph D 477 First Ave , New York City *Associate Professor of Chemistry, New York University College of Medicine* (2, 1933)
- Levy, Robert L , M D 730 Park Ave , New York City *Professor of Clinical Medicine, College of Physicians and Surgeons, Columbia University* (3, 1915)
- Lewey, F H , M D 3100 Spruce St , Philadelphia 4, Pa *Professor of Neuroanatomy, University of Pennsylvania Graduate School of Medicine, Associate in Neuropathology and Neurosurgery, Medical School* (1, 1937)
- Lewis, Gladys Kinsman, M A , Ph D 101 S Lafayette St , Denver 9, Colo (5, 1911)
- Lewis, Howard Bishop, Ph D Medical School, University of Michigan, Ann Arbor *Professor of Biological Chemistry* (1, 1925, 2, 1913, 3, 1933)
- Lewis, James C , M S , Ph D Western Regional Research Laboratory, U S Dept of Agriculture, Albany, Calif *Biochemist* (2, 1916)
- Lewis, Julian Herman, M D 4750 Champlain Ave , Chicago, Ill *Associate Professor of Pathology, University of Chicago, Member of the Otto S A Sprague Memorial Institute* (1, 1924)
- Lewis, Lena A , A B , M A , Ph D Cleveland Clinic, Euclid Ave & E 93rd St , Cleveland 6, Ohio *Research Staff* (1, 1946)
- Lewis, Robert C , Ph D 4200 E 9th Ave , Denver, Colo *Professor of Biochemistry, School of Medicine, University of Colorado* (2, 1931, 5, 1933)
- Li, Choh Hao, Ph D 4596 Life Science Bldg , University of California, Berkeley *Associate Professor of Experimental Biology* (2, 1944)
- Li, Richard C , M D National Peking University College of Medicine, Peiping, China (3, 1911)
- Libby, Raymond L , M S , Ph D Veterans Administration, Wilshire & Sawtelle Blvds , Los Angeles 25, Calif *Assoc Professor of Radio Biology, U C L A Medical Sch , Chief of Isotope Section, Veterans Hospital, Los Angeles* (6, 1938)
- Liberson, W T , M D Institute of Living, Hartford, Conn *Neurophysiologist* (1, 1948)
- Libet, Benjamin, Ph D Kabat-Kaiser Institute for Neuromuscular Rehabilitation, Permanente Hospital, Vallejo, Calif *Staff Physiologist* (1, 1912)
- Licklider, J C R , Ph D Harvard Univ , Psycho-Acoustic Lab , Cambridge, Mass *Lecturer in Psychology* (1, 1918)
- Liddell, Howard S , A M , Ph D Cornell University, Ithaca, N Y *Professor of Psychology* (1, 1925)
- Lieb, Charles C , M D 630 W 168th St , New York City *Hosack Professor of Pharmacology, College of Physicians and Surgeons Columbia University* (1R, 1936, 3, 1915)
- Lieberman, Arnold L , M D , Ph D 328 No Country Club Road, Tucson, Ariz (1, 1934)
- Lieberman, Seymour, Ph D Sloan-Kettering Institute, New York City *Associate* (2, 1948)
- Lifson, Nathan, M D , Ph D 617 Kenwood Parkway, Minneapolis, Minn *Associate Professor of Physiology, University of Minnesota Medical School* (1, 1941)
- Lightbody, Howard D , M S , Ph D QM Food and Container Institute for the Armed Forces, 1819 West Pershing Road, Chicago 9, Ill *Director, Food Labs* (2, 1936)
- Ligon, Edgar William, Jr , Ph D Production and Marketing Administration, Insecticide Division, Agriculture Bldg Washington, D C (3, 1948)
- Lilienthal, Joseph L , Jr , M D Johns Hopkins Hospital, Baltimore 5, Md *Associate Professor of Medicine* (1, 1915)
- Lillie, Ralph Stayner, Ph D , Sc D Physiological Laboratories, University of Chicago, Chicago, Ill *Professor Emeritus of Physiology* (1R, 1905, 2, 1913)
- Lillie, R D , M D Chief Pathology Laboratory, National Institute of Health, Bethesda, Md *Medical Director, U S P H S* (4, 1941)
- Lim, Robert Kho-Seng, Ph D , D Sc F R S E Academia Sinica, 320 Yo Yang Road, Shanghai, China *Director, Institute of Medicine* (1, 1923)
- Lindsley, Donald B , M A , Ph D Dept of Psychology, Northwestern Univ , Evanston, Ill *Professor of Psychology* (1, 1937)
- Linegar, Charles R , Ph D E R Squibb and Sons, Biological Laboratory, New Brunswick, N J *Chief, Biological Development and Control Laboratory* (3, 1938)
- Lineweaver, Hans, Ph D Western Regional Research Laboratory, U S Department of Agriculture, Albany, Calif *Head, Poultry Products Div* (2, 1941)
- Link, Karl Paul, Ph D Biochemistry Building, University of Wisconsin, Madison *Professor of Biochemistry* (2, 1931)
- Lintz, William, M D 36 Plaza St , Brooklyn, N Y *Late Professor of Immunology and Bacteriology and Clinical Professor of Medicine, Long Island College of Medicine* (6, 1920)
- Lipman, Mrs Miriam O , A M Presbyterian Hospital, 620 W 168th St , New York City *Research Assistant, Edward Daniels Faulkner Arthritis Clinic* (6, 1931)

- Lipmann, Fritz**, M D , Ph D Biochemical Research Laboratory, Massachusetts General Hospital, Boston *Head, Issoc Biochemist, Harvard Med Sch* (2, 1941)
- Lippincott, Stuart W**, M D University of Washington School of Medicine, Seattle, Wash *Chairman, Department of Pathology* (4, 1947)
- Lipton, Morris A**, Ph D , M D 3645 S Maryland Ave , Chicago 37, Ill *Research Associate in Medicine, University of Chicago* (2, 1946)
- Lisco, Hermann**, M D Argonne National Laboratory, Chicago 8, Ill *On leave from Harvard* (4, 1947)
- Litchfield, John T, Jr**, M D American Cyanamid Co , 1937 W Main St , Stamford, Conn *Director of Pharmacology* (3, 1940)
- Little, James Maxwell**, M S , Ph D Bowman Gray School of Medicine of Wake Forest College, Winston Salem, N C *Associate Professor of Physiology and Pharmacology* (1, 1942 3, 1947)
- Livingston, Alfred E**, Ph D Temple University School of Medicine, Philadelphia, Pa *Professor of Pharmacology* (1, 1917, 3, 1920)
- Lloyd, David P C**, D Ph Rockefeller Inst for Medical Research, 66th St and York Ave , New York 21, N Y *Associate Member* (1, 1939)
- Locke, Arthur P**, Ph D Zonite Products Corporation, New Brunswick, N J *Chief Research Chemist* (6, 1926)
- Lodholz, Edward**, M D Medical Laboratories, University of Pennsylvania, Philadelphia *Emeritus Professor of Physiology, Graduate School of Medicine* (1, 1913)
- Loeb, Leo**, M D Washington University Medical School, St Louis, Mo *Professor Emeritus of Pathology, Member, National Academy of Sciences* (1R, 1907, 4, 1913)
- Loew Earl R**, M S , Ph D Boston University School of Medicine, Boston 18, Mass (1, 1940, 3, 1946)
- Loewe, W S**, M D Department of Pharmacology, University of Utah School of Medicine, Salt Lake City 1 (3, 1936)
- Logan, Milan A**, Ph D University of Cincinnati School of Medicine Cincinnati, O *Professor of Biological Chemistry* (2, 1936)
- Long, C N H**, M Sc , D Sc , M D Yale University, New Haven, Conn *Dean Sch of Med and Sterling Prof of Physiological Chemistry* (1, 1935, 2, 1927)
- Long, Esmond R**, M D 7th and Lombard Sts , Philadelphia, Pa *Director, Henry Phipps Institute, Professor of Pathology, University of Pennsylvania* (4, 1930)
- Long, Perrin Hamilton**, M D The Johns Hopkins University, 615 N Wolfe St , Baltimore, Md *Professor of Preventive Medicine, Colonel, M C* (3, 1940)
- Longcope, Warfield T**, M D Cornhill Farm, Lee, Mass (3R, 1921, 4, 1913, 6R, 1923)
- Longenecker, Herbert Eugene**, M S , Ph D University of Pittsburgh, Pittsburgh, Pa *Dean, the Graduate School and Professor of Biochemistry* (2, 1940, 5, 1945)
- Longwell, Bernard B**, M S , Ph D 4200 East 9th Ave , Denver 7, Colo *Associate Professor of Biochemistry, Univ of Colorado, School of Medicine* (2, 1946)
- Loomis, Ted A** Ph D , M D Univ of Washington School of Medicine, Seattle, Wash *Asst Professor of Pharmacology* (3, 1948)
- Looney, Joseph M**, M D 75 Park St , West Roxbury, Mass *Director of Laboratories, V A Hospital, West Roxbury, Mass* (2, 1922)
- Loosli, Clayton Garr**, M D , University of Chicago, Chicago, Ill *Associate Professor of Medicine* (4, 1940)
- Loosli, J K**, M S , Ph D Animal Nutrition Laboratory, Cornell University, Ithaca N Y *Assoc Prof of Animal Nutr and Assoc Animal Nutritionist in Exp Sta* (5, 1944)
- Lorber, Victor**, M D , Ph D Dept of Biochemistry, Western Reserve Univ School of Medicine, Cleveland, Ohio *Associate Professor* (1, 1944)
- Lorente de N6, Rafael**, M D The Rockefeller Institute for Medical Research, 66th St and York Ave , New York City *Member* (1, 1937)
- Lorenz, Egon**, Ph D National Cancer Institute, Bethesda, Md *Biophysicist* (4, 1942)
- Loring, H S**, M A , Ph D Stanford University, Calif *Professor of Biochemistry* (2, 1938)
- Lotspeich, William D**, M D Syracuse Univ Col of Med , Syracuse, N Y *Instr in Physiology* (1, 1948)
- Loveless, Mary H**, M D New York Hospital, 525 E 68th St , New York City *Research Associate, Cornell Medical School, Physician to Out-Patients, New York Hospital* (6, 1941)
- Lowell, Francis C**, M D 65 East Newton St , Boston, Mass *Issoc Professor of Medicine, Boston Univ School of Medicine* (6, 1942)
- Lowenbach, Hans**, M D Duke University Medical School, Durham, N C *Associate Professor of Neuropsychiatry and Physiology* (1, 1946)
- Lowry, Oliver H**, M D , Ph D Washington University School of Medicine, 4580 Scott Ave , St Louis 10, Mo *Professor of Pharmacology and Head of Department* (2, 1942)
- Lubinski, Herbert**, M D Jewish General Hospital, 3755 St Catherine Rd , Montreal, Canada *Bacteriologist and Serologist* (6, 1941)
- Lucas, Colin C**, M A Sc , Ph D Banting and Best Dept of Medical Research, University of Toronto, Toronto, Canada *Associate Professor* (2, 1946)
- Lucas, George H W**, M A , Ph D University of Toronto, Toronto, Ontario, Canada *Professor of Pharmacology* (2, 1925, 3, 1928)

- Luck, James Murray**, Ph D Stanford University, Stanford University, Calif *Professor of Biochemistry* (2, 1925)
- Lucké, Baldwin**, M D 141 Montgomery Ave, Bala-Cynwyd, Pa *Professor of Pathology, University of Pennsylvania Medical School* (4, 1924)
- Luckhardt, Arno Benedict**, M S, Ph D, M D, Sc D, LL D University of Chicago, Chicago, Ill *The Dr Wm Beaumont Distinguished Service Professor of Physiology* (1, 1911)
- Ludewig, Stephan**, Ph D University of Virginia School of Medicine, University Station, Charlottesville *Associate Professor of Biochemistry* (2, 1941)
- Luduena, Froilan P**, Ph D, M D Sterling Winthrop Research Institute, Rensselaer, N Y (3, 1941)
- Lukens Francis D W**, M D University of Pennsylvania, 809 Maloney Clinic, 36th and Spruce Sts, Philadelphia *Assistant Professor of Medicine and Director, George S Cox Medical Research Institute* (1, 1938)
- Lund, E J**, Ph D Department of Zoology and Physiology, University of Texas, Austin *Professor of General Physiology* (1, 1930)
- Lundgren, Harold P**, Ph D Western Regional Research Laboratory, U S D A, Albany 6, Calif *Senior Chemist* (2, 1942)
- Lundy, John Silas**, M D The Mayo Foundation, Rochester, Minn *Chief of Section on Incesthesia* (3, 1935)
- Lurie, Max B**, M D Henry Phipps Institute, 7th and Lombard Sts, Philadelphia, Pa *Associate Professor of Experimental Pathology* (4, 1934, 6, 1930)
- Lutz, Brenton R**, Ph D Boston University, 688 Boylston St, Boston, Mass *Professor of Biology* (1, 1925)
- Luyet, Basile J**, Sc D (Biol), Sc D (Physics) St Louis University School of Medicine, St Louis, Mo *Professor of Biology* (1, 1936)
- Lyall, Harold W**, A M, Ph D Division of Laboratories and Research, New York State Department of Health, Albany *Assistant Director in charge of Antitoxin, Serum, and Vaccine Laboratories* (6, 1937)
- Lyman, Carl M**, A M, Ph D A and M College of Texas, College Station *Professor of Biochemistry and Nutrition* (2, 1940)
- Lyman, John F**, Ph D Townshend Hall, Ohio State University, Columbus *Professor of Agricultural Chemistry* (2, 1920, 5, 1933)
- Maaske, Clarence A**, Ph D University of Colorado School of Medicine, 4200 E 9th Ave, Denver, Colo *Associate Professor of Physiology and Pharmacology* (1, 1945)
- Macallum, A Bruce**, M D, Ph D Medical School, University of Western Ontario, London Ont, Canada *Res Professor of Biochemistry* (2, 1911)
- MacArthur, Edith H**, A M, Ph D RFD #1, Fort Ann, New York (5, 1933)
- MacCardle, Ross C**, Ph D National Cancer Institute, Bethesda, Md *Senior Cytologist* (1, 1918)
- MacCorquodale, D W**, M S, Ph D Abbott Laboratories, North Chicago, Ill *Head, Biochemical Research* (2, 1934)
- MacFadyen, Douglas A**, M D Presbyterian Hospital, 1753 W Congress St, Chicago, Ill *Chairman, Rush Dept of Biochemistry* (2, 1942)
- Macht, David Israel**, M D, Phar D (hon), Litt D Sinai Hospital, Baltimore, Md *Research Pharmacologist, Sinai Hospital Laboratories, and Consultant Pharmacologist, Sinai Hospital* (1R, 1916, 3, 1915)
- Macht, Martin B**, Ph D Climatic Res Lab, Lawrence, Mass *Res Physiologist* (1, 1948)
- MacKay, Eaton M**, M D The Scripps Metabolic Clinic, La Jolla, Calif (1, 1930)
- Mackenzie, Cosmo G**, Sc D Dept of Biochemistry, Cornell Univ Med College, 1300 York Ave, New York 21, N Y *Assistant Professor* (1, 1916, 2, 1916, 5, 1912)
- MacLeod, Colin M**, M D New York University College of Medicine, 477 First Ave, New York City *Professor of Bacteriology* (6, 1937)
- MacLeod, Florence L**, M A, Ph D University of Tennessee, Knoxville *Professor of Nutrition* (2, 1927, 5, 1933)
- MacLeod, Grace, M A**, Ph D 106 Morningside Drive New York City *Professor Emeritus of Nutrition Teachers College, Columbia University* (2, 1924, 5, 1933)
- MacLeod, John**, M S, Ph D Cornell University Medical College, 1300 York Ave New York City *Assistant Professor of Anatomy* (1, 1942)
- MacNabb, Andrew L**, V S, B V Sc, F A P H A Guelph, Ontario, Canada *Principal, Ontario Veterinary College* (6, 1941)
- MacNider, William deB**, M D, Sc D, LL D University of North Carolina, Chapel Hill *Kenan Research Professor of Pharmacology, Member, National Academy of Sciences* (1, 1912, 2, 1912, 3, 1909, 4, prior to 1920)
- MacPherson, Catherine F C**, M Sc, Ph D 236 Brock Ave N, Montreal, Quebec, Canada (2, 1917)
- MacPhillamy, Betty Bowser**, M S, Ph D 35 Beekman Rd, Summit, N J *Bacteriologist* (6, 1944)
- Madden, Sidney C**, M D Brookhaven, Long Island, N Y (4, 1939)
- Maddock, Stephen**, M D Boston City Hospital, Boston, Mass *Director of Surgical Research*

- Laboratory, Assistant Professor of Surgery, Tufts Medical School* (4, 1931)
- Madsen, Louis L.**, Ph D Dept of Animal Husbandry, Utah State Agricultural College, Logan Nutritionist (5, 1940)
- Magath, Thomas B.**, M S, Ph D, M D Mayo Clinic, Rochester, Minn Associate Professor of Clinical Bacteriology and Parasitology, University of Minnesota, Mayo Foundation, Consultant Physician in Clinical Laboratories, Mayo Clinic (1, 1928)
- Magill, Thomas P.**, M D Long Island College of Med, Brooklyn, N Y Prof of Bacteriology (6, 1937)
- Magoun, Horace W.**, Ph D Northwestern University Medical School, 303 E Chicago Ave., Chicago, Ill Professor of Microscopic Anatomy (1, 1937)
- Mahon, Eleanor Conway**, Ph D Iron River, Mich (4, 1940)
- Main, Rolland J.**, Ph D Eaton Laboratories, Inc, Eaton Ave, Norwich, N Y (1, 1936)
- Maison, George L.**, M S, M D Boston University Medical School, 80 E Concord St, Boston 18, Mass Professor and Head, Pharmacology Dept (1, 1939, 3, 1948)
- Major, Randolph T.**, M Sc, Ph D Merck & Co, Inc, Rahway, N J Vice President (2, 1942)
- Malkiel, Saul** Northwestern Univ Med Sch, Evanston, Ill Asst Professor of Medicine, Director, Allergy Res Lab (6, 1948)
- Mallory, G Kenneth**, M D Mallory Institute of Pathology, Boston City Hospital, Boston, Mass Professor (4, 1940)
- Mallory, Tracy B.**, M D Massachusetts General Hospital, Boston Director of Pathology and Bacteriology, Assistant Professor of Pathology, Harvard Medical School (4, 1937)
- Maloney, Arnold H.**, Ph D, M D, LL D Howard University School of Medicine, Washington, D C Professor and Head of Department of Pharmacology (3, 1932)
- Maltaner, Frank**, Ph D 388 New Scotland Ave, Albany, N Y Associate Biochemist, Division of Laboratories and Research, New York State Department of Health (6, 1920)
- Maluf, N S Rustum**, M S, Ph D 101 W Chestnut St, Louisville 2, Ky (1, 1942)
- Man, Evelyn B.**, Ph D 333 Cedar St, New Haven, Conn Assistant Professor in the Biochemistry Laboratory, Dept of Psychiatry, Yale University School of Medicine (2, 1936)
- Manery, Jeanne Forest**, M A, Ph D Medical School, University of Toronto, Toronto, Ont, Canada Demonstrator in Biochemistry (1, 1937)
- Mangun, George H.** Ph D Henry Ford Hospital, Detroit, Mich Sr Assoc in Clin Chem (2, 1947)
- Mann, Frank C.**, M A, M D, Sc D, LL D Mayo Clinic, Box 256, Rochester, Minn Director, Division of Experimental Medicine, Professor of Experimental Medicine, Mayo Foundation (1, 1916, 3, 1923, 4, 1924)
- Manning, G W.**, M D 20 Woodington Ave, Toronto, Ontario, Canada Medical Officer in Charge, No 2 R C A F Research Unit (1, 1944)
- Manville, Ira Albert**, M A, M D, Ph D 811 N W 19th Ave, Portland 9, Ore (1, 1933)
- Manwaring, Wilfred H.**, M D Stanford University, Palo Alto, Calif Professor Emeritus of Bacteriology and Experimental Pathology (4, prior to 1920, 6, 1917)
- Marcus, Stanley**, Ph D Univ of Michigan Hospital, Rackham Arthritis Res Unit, Ann Arbor, Mich Research Associate (6, 1948)
- Marine, David**, A M, M D 18 Baltimore Ave, Rehoboth, Del (1R, 1910, 4, 1913)
- Markee, Joseph E.**, Ph D Duke University School of Medicine, Durham, N C Professor of Anatomy (1, 1945)
- Markowitz, J.**, M D, Ph D University of Toronto School of Medicine, Toronto, Ont, Canada Research Associate in Physiology (1, 1929)
- Marmont, George H.**, Ph D Institute of Radiobiology and Biophysics, University of Chicago, Chicago 37, Ill, Assistant Professor of Physiology (1, 1941)
- Marmarston, Jessie** 416 N Bedford Drive, Beverly Hills, Calif Asst Professor of Medicine, Univ Southern California Staff, County Hospital and Cedars of Lebanon Hospital (6, 1932)
- Marrazzi, Amedeo S.**, M D Medical Division, Army Chemical Center, Edgewood, Md Chief, Toxicology Section (3, 1938)
- Marsh, David F.**, A B, M S, Ph D Dept of Pharmacology, West Virginia University, Morgantown, W Va Head and Associate Professor of Pharmacology (3, 1946)
- Marsh, Gordon** Ph D State University of Iowa, Iowa City Associate Professor of Zoology (1, 1944)
- Marsh, M Elizabeth**, M S, Ph D Killian Research Laboratories, 49 W 45th St, New York City Assistant Director (1 1929, 5, 1933)
- Marshak, Alfred Gordon**, M A, Ph D New York University, College of Medicine, New York City Research Associate (1, 1940)
- Marshall, Eli Kennerly, Jr.**, Ph D, M D, LL D Johns Hopkins Medical School, Baltimore, Md Professor of Pharmacology and Experimental Therapeutics Member, National Academy of Sciences (1, 1915, 2, 1913, 3, 1915)
- Marshall, Louise Hanson**, Ph D Laboratory of

- Physical Biology, Natl Insts of Health, Bethesda, Md *Physiologist* (1, 1946)
- Marshall, Wade H, Ph D National Institutes of Health, Bethesda, Md *Research Fellow, Laboratory of Physical Biology* (1, 1937)
- Martin, Arthur W, Jr, Ph D Physiology Hall, University of Washington, Seattle *Associate Professor of Physiology* (1, 1914)
- Martin, Donald S, M D Duke University School of Medicine, Durham, N C *Professor of Preventive Medicine and Public Health, Duke Hospital, Associate Professor of Bacteriology and Associate in Medicine* (6, 1913)
- Martin, Foster N, Jr Ph D, M D Department of Pharmacology, Tulane University Medical School, P O Station 20, New Orleans, La *Assistant Professor of Pharmacology* (3, 1917)
- Martin, Stephens J, M A, Ph D St Francis Hospital, Hartford, Conn (1, 1933)
- Mason, Edward C, M D, Ph D University of Oklahoma School of Medicine, Oklahoma City *Professor of Physiology* (1, 1935)
- Mason, Eleanor Dewey, A B, A M, Ph D Dept of Physiology and Nutrition, Women's Christian College, Cathedral P O, Madras, India *Professor of Physiology and Nutrition* (1, 1916)
- Mason, Herman C 3322 W Polk St, Chicago, Ill *Consulting Bacteriologist and Immunologist* (6, 1948)
- Mason, Harold L, M A, Ph D Mayo Clinic, Rochester, Minn *Professor of Physiological Chemistry, The Mayo Foundation* (2, 1941)
- Mason, Karl Ernest, Ph D The University of Rochester, School of Medicine and Dentistry, Rochester, N Y *Professor of Anatomy* (5, 1941)
- Mason, Morton F, Ph D Parkland Hospital, Oak Lawn Ave, Dallas, Texas *Professor of Pathological Chemistry and Experimental Medicine, Southwestern Medical College* (2, 1938)
- Massengale, Oliver N, Ph D Mead Johnson & Co, Research Laboratory, Evansville, Ind *Research Biochemist* (2, 1937)
- Masson, Georges M C, Ph D Cleveland Clinic Foundation, Research Division, Cleveland, Ohio (1, 1944)
- Mathews, Albert P, Ph D, D Sc (hon) Marine Biological Laboratory, Woods Hole, Mass *Emeritus Carnegie Professor of Biochemistry, Univ of Cincinnati* (1R, 1898, 2, 1906)
- Matson, Gustave A, Ph D Latter Day Saints Hospital Blood Bank, Salt Lake City 3, Utah *Serologist and Bacteriologist, Director of Blood Bank* (6, 1946)
- Matthews, Samuel A, Ph D Williams College, Williamstown, Mass *Professor of Biology* (1, 1948)
- Mattill, Henry A, A M, Ph D State University of Iowa, Iowa City *Professor and Head of Department of Biochemistry* (1, 1913, 2, 1909, 5, 1933)
- Mattis, Paul A, B S, D Sc Atomic Energy Medical Research Project, Western Reserve University, Cleveland, Ohio *Section Chief, Pharmacology* (3, 1916)
- Maurer, Frank W, Ph D 301 Lake Ave, Newton Highlands 61, Mass (1, 1911)
- Mautz, Frederick R, M D Western Reserve School of Medicine, Cleveland 6, O *Assistant Professor of Surgery* (1, 1915)
- Maver, Mary E, Ph D National Cancer Institute, Bethesda 14, Md *Senior Biochemist* (2, 1917)
- Mavor, James Watt, Ph D 8 Gracewood Park, Cambridge, Mass (1, 1930)
- Maxfield, Mary E, A M, Ph D University of Tennessee Medical College, Dept of Pharmacology, Memphis, Tenn (1, 1947)
- Mayer, Manfred M, Ph D 1739 Lutaw Place, Baltimore, Md *Assoc Professor of Bacteriology, Johns Hopkins Hospital, School of Hygiene* (6, 1916)
- Mayerson, Hymen S, Ph D Tulane University School of Medicine, Station 20, New Orleans, La *Professor of Physiology and Head of Dept of Physiology* (1, 1928)
- Maynard, L A, Ph D, Sc D Cornell University, Ithaca, N Y *Professor of Nutrition and Biochemistry, Director, School of Nutrition, Member National Academy of Sciences* (2, 1930, 5, 1933)
- Mazur, Abraham, Ph D College of the City of New York, 140th St and Convent Ave, New York City, *Asst Professor* (2, 1944)
- McCann, William S, M D, D Sc (Hon) University of Rochester, School of Medicine, Rochester, N Y *The Charles A Dewey Professor of Medicine* (2, 1923, 5, 1933)
- McCarrell, June D Department of Biology, Hood College, Frederick, Md (1, 1942)
- McCarty, Maclyn, M D 66th Street and York Avenue, New York 21, N Y *Associate, Rockefeller Institute for Medical Research* (6, 1947)
- McCawley, Elton Leeman, Ph D Yale Medical School, New Haven, Conn *Instructor in Pharmacology* (3, 1944)
- McCay, Clive M, M S, Ph D Animal Nutrition Laboratory, Dairy Building, Cornell University, Ithaca, N Y *Professor of Nutrition* (2, 1929, 5, 1933)
- McChesney, Evan William, Ph D Ladox Laboratories, Inc, 2 Vine St, Philadelphia, Pa (1, 1944)
- McClellan, Walter S, M D Saratoga Sprs, Saratoga Springs, N Y *Medical Director, Associate Professor of Medicine, Albany Medical College* (1, 1931)
- McClendon, J F, M S, Ph D Route 1, Box 383, Trooper Road, Norristown, Pa *Research Pro-*



- fessor of Physiology, Hahnemann Medical College (1, 1910, 2, 1914, 5, 1935)
- McClung, L S Indiana Univ, Bloomington, Ind Chairman and Assoc Professor, Dept of Bacteriology (6, 1948)
- McCullum, Elmer Verner, Ph D, Sc D, LL D Johns Hopkins University, Baltimore, Md Professor Emeritus of Biochemistry Member, National Academy of Sciences (2, 1910, 5, 1933)
- McCullum, Ernestine Becker, M A, Johns Hopkins University, School of Hygiene, Baltimore 5, Md Assistant Professor of Biochemistry (5, 1938)
- McCouch, Grayson Prevost, M D University of Pennsylvania, Philadelphia Assistant Professor of Physiology (1, 1925)
- McCouch, Margaret Sunwalt, M S, Ph D University of Pennsylvania Medical School, Philadelphia (1, 1934)
- McCoy, Richard H, Ph D Univ of Pittsburgh, Pittsburgh, Pa Assoc Research Professor of Chemistry (5, 1948)
- McCrea, Forrest D, Ph D Duke University School of Medicine, Durham, N C Associate Professor of Physiology and Pharmacology (1, 1929, 3, 1937)
- McCrudden, Francis H, M D 19 Stoneleigh Road, West Newton, Mass Assistant Medical Director, New England Mutual Life Insurance Co (2, 1906)
- McCullagh, D Roy, Ph D, FIC 86 Orange St, Bloomfield, N J Director, Dept of Biochemistry Schering Corp (2, 1932)
- McCulloch, Warren Sturgis, M A, M D University of Illinois, College of Medicine, 912 S Wood St, Chicago Associate Professor of Psychiatry (1, 1936)
- McCutcheon, Morton, M D University of Pennsylvania Medical School, Philadelphia Professor of Pathology (4, 1925)
- McDonald, Francis Guy, M S, Ph D Research Laboratory, Mead Johnson & Co, Evansville Ind Assistant Director of Research (2, 1936, 5, 1947)
- McElroy, L W Dept of Animal Science, University of Alberta Edmonton, Canada Associate Professor of Animal Husbandry (5, 1944)
- McElroy, William D, Ph D Dept of Biology, Johns Hopkins University, Baltimore, Md Assistant Professor of Biology (1, 1945)
- McElroy, William Swindler, M D School of Medicine, University of Pittsburgh, Pittsburgh, Pa Professor of Physiological Chemistry, Dean, School of Medicine (2 1919)
- McFarland, Ross A, Ph D Harvard University, School of Public Health, Boston, Mass (1, 1943)
- McFarlane, William Douglas, Ph D 496 Queen St, E, Toronto, Canada Director of Research, Canadian Breweries, Ltd (2, 1933)
- McGinty, Daniel A, M A, Ph D Parke, Davis & Co, Detroit, Mich Research Physiologist (1, 1925)
- McGuigan, Hugh Ahster, Ph D, M D Univ of Illinois College of Medicine, Chicago Emeritus Professor of Pharmacology (1R, 1907, 2, 1906, 3R, 1913)
- McHargue, J S, M S Ph D, D Sc Department of Chemistry, Kentucky Agricultural Experiment Station, University of Kentucky, Lexington Emeritus Member (2, 1927)
- McHenry, E W, M A, Ph D, F R S C School of Hygiene, University of Toronto, Toronto, Canada Professor of Public Health Nutrition (2 1938 5, 1935)
- McIntyre, A R, Ph D, M D College of Medicine, University of Nebraska, 42nd and Dewey Ave, Omaha Professor of Physiology and Pharmacology (1, 1933, 3, 1938)
- McKee, Albert P State Univ of Iowa, Iowa City, Iowa Assoc Professor of Bacteriology (6, 1948)
- McKee, Clara M, Squibb Institute for Medical Research, New Brunswick, N J Associate in Microbiology (6, 1941)
- McKee, Frank W, M D University of Rochester School of Medicine and Dentistry, Rochester, N Y Instructor in Pathology and Rockefeller Foundation Fellow (4, 1947)
- McKee, Ralph Wendell, M S Ph D Harvard Medical School, 25 Shattuck St, Boston, Mass Assistant Professor of Biochemistry (2, 1946)
- McKennis, Herbert, Jr, Ph D Johns Hopkins School of Medicine, Baltimore, Md Instructor in Physiological Chemistry (2, 1948)
- McKibbin, John M, Ph D Syracuse Univ College of Medicine, Syracuse, N Y Assoc Professor of Biochemistry (2, 1948)
- McLain, Paul L, M D University of Pittsburgh Medical School, Pittsburgh, Pa Assistant Professor of Physiology and Pharmacology Major, M C (1, 1948, 3, 1940)
- McLean, Franklin C, Ph D, M D University of Chicago, Chicago, Ill Professor of Pathological Physiology (1, 1914, 2, 1916, 3, 1916)
- McLean, I William, Jr, B S, M D Virus Research Division, Parke-Davis Laboratory, Detroit, Mich Research Virologist (6, 1946)
- McLester, James S, M D, LL D University of Alabama, 930 S 20th St, Birmingham Professor of Medicine (5, 1933)
- McManus, J F A, M D Medical College of Alabama, Birmingham, Ala Asst Professor of Pathology (4, 1948)
- McMaster, Philip D, M D The Rockefeller Institute for Medical Research, 66th St and York Ave, New York City (1, 1924)

- McMeekin, Thomas L**, Ph D Eastern Regional Research Laboratory, U S Department of Agriculture, Philadelphia, Pa *Head of Protein Div* (2, 1935)
- McNamara, Bernard P**, M S, Ph D Pharmacology Section, Medical Division, Army Chemical Center, Md *Pharmacologist* (3, 1947)
- McNaught, James Bernard**, M D University of Colorado School of Medicine, Denver 7 *Professor and Head of Dept of Pathology* (4, 1936)
- McPhail, Murchie K**, Ph D National Defense Board, Suffield Experimental Station, Suffield, Alberta, Canada (3, 1941)
- McQuarrie, Irvine**, Ph D, M D University of Minnesota, Minneapolis *Professor and Head of Department of Pediatrics* (4, 1927, 5, 1933)
- McShan, W H**, M A, Ph D Biology Building, University of Wisconsin, Madison 6, Wis *Associate Professor of Zoology* (2, 1917)
- Medes, Grace**, Ph D Lankenau Hospital Research Institute, Philadelphia, Pa *Research Physiological Chemist* (2, 1930)
- Medlar, Edgar M**, M D Path Bldg, Room 703 Bellevue Hospital, 1st Ave at 26th St, New York, N Y *Pathologist* (4, 1927)
- Meek, Walter J**, Ph D University of Wisconsin, Madison *Professor of Physiology, Associate Dean of the Medical School, Member of National Academy of Sciences* (1, 1908)
- Mehl, John W**, Ph D Dept of Biochemistry, University of Southern California, Los Angeles, Calif *Professor of Biochemistry* (2, 1946)
- Meiklejohn, Gordon**, M D, C M Univ of California Med Sch, Berkeley, Calif *Instructor in Medicine* (6, 1948)
- Mellon, Ralph R**, M D, M Sc, Dr P H, Sc D (hon) Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh *Director* (6, 1918)
- Melnick, Daniel**, Ph D Quartermaster Food and Container Institute for the Armed Forces, Chicago 9, Ill *Chief of the Food Development Division* (2, 1940, 5, 1942)
- Melnick, Joseph L**, Ph D Yale University School of Medicine, New Haven, Conn *Res Assoc of Preventive Medicine* (2, 1946, 6, 1948)
- Melville, Donald B**, M S, Ph D Cornell University Medical College, New York City *Assoc Professor of Biochemistry* (2, 1947)
- Melville, Kenneth Ivan**, M Sc, M D, C M McGill University, Montreal, Canada *Assistant Professor of Pharmacology* (3, 1931)
- Mendel, Bruno**, M D University of Toronto, 100 College Street, Toronto 5, Canada *Professor of Cellular Physiology, Banting-Best Department of Medical Research* (2, 1917)
- Mendenhall, Walter L**, S M, M D 9 Acadia St, Cambridge, Mass *Professor of Pharmacology, retired, Boston University Medical School* (1, 1915, 3, 1917)
- Mendez, Rafael**, M D National Institute of Cardiology, Calzada de la Piedad 300, México D F, México *Head of the Dept of Pharmacology* (3, 1911)
- Meneely, George R**, M D Thayer Veterans Administration Hospital, Nashville, Tenn *Assistant Professor of Medicine* (4, 1946)
- Menkin, Vally, M A**, M D Temple Univ School of Medicine, Philadelphia, Pa *Associate Professor of Experimental Pathology* (1, 1932, 4, 1932, 6, 1931)
- Menten, Maud L**, M D, Ph D University of Pittsburgh, Pittsburgh, Pa *Associate Professor of Pathology* (1, 1915, 4, 1927)
- Mettier, Stacy R**, M D University of California Hospital, San Francisco *Associate Professor of Medicine* (4, 1932)
- Mettler, Fred A**, A M, Ph D, M D Department of Neurology, College of Physicians and Surgeons, Columbia University, New York City *Associate Professor of Anatomy* (1, 1937)
- Meyer, Arthur E**, Ph D Fellows Pharmaceutical Lab, New York City (1, 1948)
- Meyer, Curtis E**, M S, Ph D The Upjohn Co, Kalamazoo, Mich *Senior Research Chemist* (2, 1912)
- Meyer, Karl M D**, Ph D 630 W 168th St, New York City *Associate Professor of Biochemistry, Dept of Medicine, College of Physicians and Surgeons Columbia University* (2, 1934)
- Meyer, Karl F**, M D, Ph D George W Hooper Foundation, Univ of California Medical Center, San Francisco *Director, George W Hooper Foundation* (4, 1930, 6, 1922)
- Meyerhof, Otto**, M D, LL D Department of Physiological Chemistry, University of Pennsylvania School of Medicine, Philadelphia *Research Professor of Biochemistry* (2, 1941)
- Michaelis, Leonor**, M D, LL D Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Member Emeritus* (2, 1929)
- Mickelsen, Olaf**, Ph D University of Minnesota, Department of Physiological Hygiene, Stadium South Tower, Minneapolis *Associate Professor* (2, 1944)
- Mider, George Burroughs**, M D Strong Memorial Hospital, Rochester 7, N Y *Professor of Cancer Research* (4, 1940)
- Miles, Walter R**, A M, Ph D 333 Cedar St, New Haven, Conn *Professor of Psychology,*

- The School of Medicine and the Institute of Human Relations, Yale University, Member of the National Academy of Sciences* (1, 1919)
- Milhorat, Ade T, M D Cornell University Medical College, New York 21, N Y *Asst Professor of Medicine and Instructor in Pharmacology, Research Fellow, Russell Sage Institute of Pathology* (1, 1934, 3, 1937, 5, 1935)
- Miller, Augustus Taylor, Jr, Ph D University of North Carolina Medical School, Chapel Hill *Associate Professor of Physiology* (1, 1944)
- Miller, Benjamin F, Ch E, M D Dept of Medicine, Univ of Chicago, Chicago, Ill *Assistant Professor of Medicine* (2, 1938)
- Miller, Carey D, M S University of Hawaii, Honolulu *Professor of Food and Nutrition, Hawaii Agricultural Experimental Station* (5, 1942)
- Miller, C Phillip, M D, M S University of Chicago, Chicago, Ill *Professor of Medicine* (4, 1925, 6, 1928)
- Miller, Edgar G, Jr, Ph D 630 W 168th St, New York City *Professor of Biological Chemistry, Columbia University* (2, 1930)
- Miller, Franklin R, M D Jefferson Medical College and Hospital, Division of Hematology, Philadelphia, Pa *Associate Professor of Medicine* (4, 1940)
- Miller, Frederick R, A M, M D, F R C P (C), F R S Faculty of Medicine, University of Western Ontario, London, Ont, Canada *Research Professor of Neurophysiology* (1, 1908)
- Miller, G H, M D American College of Surgeons, 40 E Erie St, Chicago 11, Ill *Director of Educational Activities* (3, 1925)
- Miller, George A, Ph D Harvard Univ, Psycho-Acoustic Lab, Cambridge, Mass *Res Fellow* (1, 1948)
- Miller, H R, M D 1020 Park Ave, New York 28, N Y Montefiore Hospital, New York City (1, 1947)
- Miller, Leon L, Ph D, M D University of Rochester Sch of Med and Dentist, Rochester, N Y *Assoc Prof of Radiobiology and Biochemistry* (2, 1947)
- Miller, Lila, M S, Ph D Dept of Biological Chemistry, University of Michigan, Ann Arbor, Mich *Assistant Professor of Biological Chemistry* (2, 1946)
- Miller Lloyd C, Ph D Sterling Winthrop Research Institute, 33 Riverside Ave, Rensselaer, N Y *Director, Biology Division* (3, 1938)
- Miller, R C, Ph D Pennsylvania State College, State College *Assistant Professor Agricultural and Biological Chemistry* (5, 1935)
- Miller, Zelma Baker, Ph D George Washington Sch of Med, Washington, D C *Res Assoc, Dept of Pharmacology* (2, 1940)
- Mills, Clarence A, Ph D, M D Cincinnati General Hospital, Cincinnati 29, Ohio *Professor of Experimental Medicine, University of Cincinnati* (1, 1921, 2, 1921)
- Minot, Annie Stone, Ph D Vanderbilt University Medical School, Nashville, Tenn *Research Associate, Department of Biochemistry* (1, 1923)
- Mirsky, Alfred E, Ph D Rockefeller Inst, 66th St and York Ave, New York 21, N Y *Associate Member* (2, 1941)
- Mirsky, I Arthur, M Sc, M D, C M The Jewish Hospital, Cincinnati, O *Director, The May Institute for Medical Research, Assistant Professor of Biochemistry, University of Cincinnati* (1, 1936)
- Mitchell, Harold H, M D 120 Lasky Drive, Beverly Hills, Calif (6, 1943)
- Mitchell, Harold H, M S, Ph D 555 Davenport Hall, University of Illinois, Urbana, Ill *Professor of Animal Nutrition* (2, 1919, 5, 1933)
- Mitchell, Helen S, Ph D University of Massachusetts, Amherst, Mass *Dean of the School of Home Economics* (2, 1925, 5, 1933)
- Mitchell, Philip H, Ph D Brown University Providence 12, R I *Robert P Brown Professor of Biology* (2, 1909)
- Modell, Walter, M D Cornell University Medical College, 1300 York Ave, New York, N Y *Instructor in Pharmacology* (3, 1944)
- Moe, Gordon Kenneth, Ph D, M D University of Michigan, Ann Arbor *Associate Professor of Pharmacology* (3, 1944)
- Mohn, James F, M D 24 High St, Buffalo, N Y *Asst Professor in Bacteriology and Immunology Univ of Buffalo School of Medicine* (6, 1946)
- Molitor, Hans, M D Merck Institute for Therapeutic Research, Rahway, N J *Director* (1, 1933, 3, 1942)
- Molland, Jacob, M D, Ph D Univ of Oslo, Oslo, Norway *Professor of Pharmacology* (3, 1948)
- Molomut, Norman, M A, Ph D Biological Labs, 16 Clinton St, Brooklyn 2, N Y *Director of Research and Director of Laboratories* (6, 1942)
- Moon, Virgil H, M Sc, M D Jefferson Medical College, Philadelphia, Pa *Professor of Pathology* (4, 1934)
- Moore, A R, Ph D University of Oregon, Eugene *Research Professor of General Physiology in the Department of Psychology* (1, 1912)
- Moore, Carl Vernon, M D Washington University School of Medicine, St Louis, Mo *Professor of Medicine* (4, 1938, 5, 1941)
- Moore, Dan H, Ph D Columbia Univ Coll of Phys and Surg, New York City *Assoc Professor of Anatomy* (1, 1948)
- Moore, Lane A, Ph D Division of Nutrition and Physiology, Bureau of Dairy Industry, U S

- Dept of Agriculture, Beltsville, Md *Head, Section of Dairy Cattle Nutrition* (5, 1940)
- Moore, Robert A**, M D Washington University Medical School, St Louis, Mo *Dean and Professor of Pathology* (4, 1929)
- Moore, Robert M**, M D University of Texas Medical School, Galveston, Tex (1, 1932)
- Moorhouse, Victor Henry K**, M B University of Manitoba, Winnipeg, Canada *Professor of Physiology* (1, 1912)
- Morehouse, Laurence E**, M Ed, Ph D University of Southern California, Los Angeles 7 *Associate Professor* (1, 1947)
- Morgan, Agnes Fay**, M S, Ph D University of California, Berkeley *Professor of Home Economics, Biochemist, Agric Exp Station* (2, 1929, 5, 1933)
- Morgan, Charles F**, Ph D Georgetown University School of Medicine, Washington, D C *Professor of Physiology and Chairman of the Department of Physiology* (1, 1918, 3, 1917)
- Morgan, Clifford T**, M A, Ph D Psychology Department, Johns Hopkins University, Baltimore 18, Md (1, 1913)
- Morgan, Isabel M**, Ph D Poliomyelitis Research Center, Baltimore 1, Md *Asst Professor of Epidemiology, Johns Hopkins Univ* (6, 1947)
- Morgulis, Sergius**, A M, Ph D University of Nebraska College of Medicine, Omaha *Professor of Biochemistry* (1, 1914, 2, 1916)
- Morison, Robert S**, M D Rockefeller Foundation, 66th St and York Ave, New York City *Asst Director of the Med Sciences* (1, 1938)
- Moritz, Alan R**, M D Harvard Medical School, Boston, Mass *Professor of Legal Medicine* (4, 1934)
- Morrell, Clarence Allison**, M A, Ph D Department of Pensions and National Health, Laboratory of Hygiene, Sussex and John Sts, Ottawa, Canada *Senior Pharmacologist* (3, 1937)
- Morris, Harold P**, M S, Ph D National Cancer Institute, Bethesda, Md *Principal Biochemist in Nutrition* (2, 1944, 5, 1943)
- Morris, Marion C**, Ph D Public Health Research Institute of City of New York, Foot of East 15th St, New York City *Associate in Division of Infectious Diseases* (6, 1936)
- Morrison, Dempsey B**, M S, Ph D University of Tennessee College of Medicine, Memphis *Associate Professor of Chemistry* (2, 1936)
- Morrison, James L**, Ph D Emory University School of Medicine, Emory University, Ga *Assistant Professor of Pharmacology* (3, 1944)
- Morse, Minerva**, M S, Ph D 5525 Kimbark Ave, Chicago, Ill *Research Associate, Department of Pediatrics, University of Chicago* (2, 1934)
- Morse, Withrow**, Ph D 32 Manchester Rd, Eastchester, via Tuckahoe, N Y *Consultant* (2, 1914)
- Mortimer, Bernard**, Ph D, M D 25 N Ottawa St, Joliet, Illinois, Cook County Hospital, Chicago (1, 1936)
- Morton, John J**, M D University of Rochester, School of Medicine and Dentistry, Rochester, N Y *Professor of Surgery* (4, 1927)
- Moses, Campbell**, M D Univ of Pittsburgh Sch of Med, Pittsburgh, Pa *Director, Addison H Gibson Lab of Applied Physiology* (1, 1918)
- Motley, H L**, Ph D, M D Jefferson Hospital, Philadelphia, Pa *Director, Cardio-Respiratory Lab Jefferson Med Coll Assoc Professor of Medicine* (1, 1917)
- Moulton, C Robert**, Ph D 5602 Dorchester Ave, Chicago 37, Ill (5, 1933)
- Moxon, Alvin L**, Ph D College Station, Brookings, S D *Head, Chemistry Department, South Dakota Agricultural Experiment Station* (2, 1911)
- Moyer, Arden W**, Ph D 107 Mountain View Rd, Englewood, N J *Research Biochemist, Lederle Labs, Pearl River, N Y* (6, 1916)
- Moyer, Carl A**, Ph D 6417 Glenrose Ct, Dallas 1, Texas Southwestern Medical College Dallas *Professor of Experimental Surgery* (1, 1913)
- Mudd, Stuart**, M A M D University of Pennsylvania, Philadelphia *Professor of Bacteriology* (1, 1921, 4, 1927, 6, 1927)
- Muehlberger, Clarence W**, M S, Ph D State Health Department Laboratories Lansing, Mich *State Toxicologist* (3, 1928)
- Mueller, J Howard**, M S, Ph D Harvard Medical School, Boston, Mass *Charles Wilder Professor of Bacteriology and Immunology* (2, 1922, 4, 1927, 6, 1920)
- Mukherji, B**, M B, D Sc All-India Institute of Hygiene and Public Health, Calcutta *Director, Biochemical Standardization Laboratory* (3, 1938)
- Mulder, Arthur G**, Ph D University of Tennessee College of Medicine, Memphis *Associate Professor of Physiology* (1, 1937)
- Mulford, Dwight J**, Ph D 306 Riverway, Boston, Mass *Chief of Laboratory, Massachusetts Dept of Public Health, Associate in Physical Chemistry, Harvard University* (2, 1948)
- Mulinos, M G**, M D, Ph D New York Medical College, Flower and Fifth Avenue Hospitals, Fifth Ave and 105th St, New York 29, N Y *Associate Professor of Pharmacology* (3, 1931)
- Mull, James W**, Ph D 2020 State St, Quincy, Ill *Associate, Quincy Specialties Co* (2, 1937)
- Muller, Otto H**, R N Dr Department of Physiology, Syracuse University College of Medicine, Syracuse 10, N Y (1, 1947)
- Mulligan, Richard M**, M D University of Colorado School of Medicine, 4200 East 9th Ave

- nue, Denver, Colo *Associate Professor of Pathology* (4, 1947)
- Mullin, F J, M S, Ph D *Physiology Department, University of Chicago, Chicago 37, Ill Assistant Professor of Physiology* (1, 1937)
- Munro, F L, Ph D *Jefferson Medical College, Philadelphia, Pa Research Chemist* (2, 1948)
- Munro, Muriel Platt, Ph D *Jefferson Medical College, Philadelphia, Pa Research Chemist* (2, 1948)
- Munsell, Hazel E, M A, Ph D *Nutrition Biochemistry Labs, Dept of Food Technology, Mass Inst of Technology, Cambridge Research Associate* (5, 1933)
- Muntwyler, Edward, Ph D *Long Island College of Medicine, 350 Henry St, Brooklyn, N Y Professor of Biochemistry* (2, 1931)
- Muntz, John A, Ph D, 3356 Euclid Heights Blvd, Cleveland Heights, Ohio *Asst Professor Western Reserve University* (2, 1948)
- Murlin, John R, A M, Ph D, Sc D *University of Rochester Medical School, 260 Crittenden Blvd, Rochester, N Y Professor Emeritus of Physiology and Director Emeritus of Department of Vital Economics* (1R, 1906, 2, 1908, 5, 1933)
- Murphy, James B, M D *Rockefeller Institute for Medical Research, 66th St and York Ave, New York City Member* (4, prior to 1920)
- Murray, Everitt G D, O B E, B A honors in Natural Science, M A, L M S S A, F R S C *McGill University, Montreal, Canada Professor of Bacteriology and Immunology and Head of the Department, McGill University, Bacteriologist-in-Chief to the Royal Victoria Hospital, to the Children's Memorial Hospital and to the Alexandra Hospital* (6, 1933)
- Mushett, Charles W, Ph D *Merck Institute for Therapeutic Research, Rahway, N J Head, Department of Pathology* (4, 1948)
- Muus, Jytte, Mag Scient (Univ of Copenhagen) *Mount Holyoke College, South Hadley, Mass Associate Professor* (2, 1946)
- Myers, Chester N, Ph D, Sc D 34 Cedar Place, Yonkers 5, N Y *Chief, Division Chemotherapy, N Y Skin and Cancer Hospital, Associate in Dermatology and Syphilology, College of Physicians and Surgeons, Research Chemist, Vanderbilt Clinic, Director, Chemical and Clinical Research, H A Metz Laboratories, Inc* (2, 1922)
- Nachmansohn, David, M D *Dept of Neurology, College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York City Research Associate in Neurology* (1, 1940)
- Nadler, J Ernest, M D, Med D Sc 80-16 Leferts Blvd, Kew Gardens 15, N Y (3, 1940)
- Nahum, Louis N, M D 1142 Chapel St, New Haven, Conn *Assistant Professor of Physiology, Yale University* (1, 1934)
- Najjar, Victor A, M D *Unit for Microbiology, Univ of Cambridge, Cambridge, Eng Nat'l Res Council Fellow* (2, 1946)
- Nash, Thomas P, Jr, M A, Ph D 875 Monroe Ave, Memphis, Tenn *Professor of Chemistry, College of Medicine, Dean of School of Biological Sciences, University of Tennessee* (2, 1923)
- Nasset, Edmund S, M S, Ph D *University of Rochester, 260 Crittenden Blvd, Rochester, N Y Professor of Physiology* (1, 1932, 5, 1940)
- Nathanson, Ira T, M S, M D *Massachusetts General Hospital, Boston Instructor in Surgery, Harvard Medical School, Assistant in Surgery, Mass General Hospital* (1, 1943)
- Nathanson, Morris D, M D 658 S Bonnie Brae St, Los Angeles, Calif *Associate Clinical Professor of Medicine, University of Southern California School of Medicine* (3, 1940)
- Necheles, Heinrich, M D, Ph D *Michael Reese Hospital, Chicago, Ill Director, Dept of Gastro-intestinal Physiology, Michael Reese Hospital, Professorial Lecturer in Physiology, University of Chicago* (1, 1929)
- Neill, James M, Ph D *Medical College, Cornell University, 1300 York Ave, New York City Professor of Bacteriology and Immunology* (6, 1930)
- Neilson, Charles Hugh A M, Ph D, M D *Humboldt Building, St Louis, Mo Associate Dean and Professor of Medicine St Louis University Medical School* (2, 1906)
- Nelson, Arthur A M D, Ph D *Food and Drug Administration, Federal Security Agency, Washington, D C Senior Pathologist, Division of Pharmacology* (4, 1942)
- Nelson, Carl Ferdinand, M D, Ph D *Department of Biochemistry, University of Kansas, Lawrence Professor of Physiological Chemistry* (2, 1914)
- Nelson, Carl T, A B, M A, M D *College of Physicians and Surgeons, 630 West 168th St, New York 32, N Y Instructor in Dermatology* (6, 1943)
- Nelson, Erwin E Ph D, M D *Drug Division, Food & Drug Administration, F S A, Washington, D C Chief, New Drug Section, Adjunct Clinical Professor of Pharmacology, George Washington Univ Sch of Medicine* (1, 1923, 3, 1924)
- Nelson, E M, M S, Ph D *Food and Drug Administration Federal Security Agency, Washington 25, D C Chief, Vitamin Division* (2, 1927, 5, 1933)
- Nelson, John B, Ph D *Rockefeller Institute for Medical Research, Princeton, N J Associate Member* (4, 1934)
- Nelson, John M, Ph D *Columbia University, New York City Professor of Organic Chemistry* (2, 1923)
- Nelson, Norton, Ph D *New York University College of Medicine, 477 First Ave, New York*

- 16, N Y Associate Professor of Industrial Medicine (2, 1946)
- Nelson, P Mabel, M S, Ph D Iowa State College, Ames Dean, Division of Home Economics (5, 1934)
- Nelson, Tell, M A, M D 1415 Kalakaua Ave, Honolulu 19, T H Practicing Physician (6, 1938)
- Nelson, Victor E, M S Iowa State College, Ames Professor of Physiological Chemistry (2, 1924)
- Nelson, Warren O, M S, Ph D Dept of Anatomy, School of Medicine, Univ of Iowa, Iowa City Professor of Anatomy (1, 1937)
- Neter, Erwin, M D Children's Hospital, 219 Bryant St, Buffalo, N Y Attending Bacteriologist (6, 1937)
- Nettleship, Anderson, M D University of Arkansas School of Medicine, Little Rock Professor and Head of Department of Pathology (1, 1942)
- Neuberg, Carl, Ph D, M D (h c), Med Chem D (h c), Biol D (h c), Dr Eng (h c), LL D 536 W 113th St, New York 25, N Y Research Professor, New York University, Member or hon member of the Academies of Science of Copenhagen, Göttingen, Leningrad, Lisbon, Lund, Prag, Rome and Upsala (2, 1941)
- Neumann, Charles, M D 525 East 68th Street, New York 21, N Y Resident Surgeon, New York Hospital, Instructor in Surgery, Cornell University Medical College (1, 1941)
- Neurath, Hans, Ph D School of Medicine, Duke University, Durham, N C Professor of Physical Biochemistry (2, 1940, 6, 1944)
- Neuwelt, Frank, M D 504 Broadway, Garv Ind Research Associate, Department of Gastrointestinal Research, Michael Reese Hospital (1, 1940)
- Neuwirth, Isaac, Ph D 209 E 23rd St, New York City Professor of Pharmacology, New York University College of Dentistry (2, 1924, 3, 1931)
- Newman, Elliot V, M D Johns Hopkins Hosp, Baltimore, Md Asst Professor of Med (1, 1948)
- Nice, Leonard B, Ph D Chicago Medical School, 710 S Wolcott Ave, Chicago, Ill Professor of Physiology and Pharmacology (1, 1921)
- Nicholas, John S, M S, Ph D Osborn Zoological Laboratory, Yale University, New Haven, Conn Bronson Professor of Comparative Anatomy (1, 1927)
- Nicholson, Hayden C, M S, M D Division of Medical Sciences, National Research Council, 2101 Constitution Ave, Washington 25, D C (1, 1932)
- Nickerson, John L, Ph D Columbia University, 630 W 168th St, New York 32, N Y Associate Professor of Physiology (1, 1945)
- Nickerson, Mark, Sc M, Ph D University of Utah School of Medicine, Salt Lake City, Utah Assistant Professor of Pharmacology (3, 1947)
- Nicolet, Ben H, Ph D Bureau of Dairy Industry, U S Department of Agriculture, Beltsville, Md Senior Chemist (2, 1932)
- Nicoll, Paul A, Ph D Indiana University, Bloomington Assistant Professor of Physiology (1, 1915)
- Niemann, Carl G, Ph D California Institute of Technology, Pasadena 4, Calif Professor, Organic Chemistry (2, 1940)
- Nigg, Clara, M A, Ph D c/o E R Squibb & Sons, New Brunswick, N J Head, Dept of Bacteriology and Virology, Dept of Microbiological Development (6, 1929)
- Nims, Leslie F, M A, Ph D Biology Department, Brookhaven National Laboratory, Upton, Long Island, N Y Acting Head of Department (1, 1940)
- Noble, Robert Laing, M D, Ph D D Sc Department of Medical Research, University of Western Ontario, London, Ontario (1, 1941)
- Nord, F F, Ph D Fordham University, Dept of Organic Chemistry, New York City Professor of Chemistry (2, 1940)
- Norris, Earl R, Ph D University of Washington, Seattle Professor of Biochemistry and Executive Officer (2, 1938)
- Norris, L C, Ph D Rice Hall, Cornell University, Ithaca, N Y Professor of Nutrition (2, 1939, 5, 1934)
- Northrop, J H, M A, Ph D, Sc D, LL D Rockefeller Institute for Medical Research, Princeton, N J Member (2, 1938)
- Northup, David W, M A, Ph D West Virginia University Medical School, Morgantown Associate Professor of Physiology (1, 1936)
- Novy, F G, M D, Sc D, LL D 721 Forest Ave, Ann Arbor, Mich Dean Emeritus of the Medical School and Professor Emeritus of Bacteriology, University of Michigan, Member, National Academy of Sciences (2, 1906)
- Nowinski, W W, Ph D Univ of Texas, Sch of Med, Galveston, Texas Res Assoc (1, 1948)
- Oberst, Fred W, M S, Ph D U S School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas Pharmacologist-Biochemist (2, 1936)
- Ochoa, Severo, M D New York University College of Medicine, 477 First Ave, New York City 16 Professor of Pharmacology (2, 1942)
- Ogden, Eric, M R C S (England), L R C P (London) University of Texas School of Medicine, Galveston Professor of Physiology and Clinical Physiologist, John Sealy Hospital (1, 1941)
- O'Hare, James P, M D 520 Commonwealth Ave, Boston, Mass Physician, Peter Bent

- Brigham Hospital, Lecturer on Medicine, Harvard Medical School (4, 1927)
- Ohlson, Margaret A, M S, Ph D Dept of Foods and Nutrition, Michigan State College, East Lansing Professor and Head, Department of Foods and Nutrition (5, 1945)
- Okey, Ruth, Ph D 1553 Life Sciences Bldg, University of California, Berkeley Professor of Home Economics and Biochemist, State Exp Station (2, 1922, 5, 1933)
- Olcott, Harold S, M S, Ph D Western Regional Research Laboratory, U S Department of Agriculture, Albany 6, Calif Principal Chemist (2, 1935)
- Oldham, Helen, M S, Ph D University of Chicago, Chicago, Ill Assistant Professor, Dept of Home Economics (5, 1946)
- Olitsky, Peter K, M D Rockefeller Institute for Medical Research, 66th St and York Ave, New York City Member (4R, 1923, 6, 1917)
- Oliver, Jean Redman, M D Hoagland Laboratory, 335 Henry St, Brooklyn, N Y Professor of Pathology, Long Island College of Medicine (1, 1924, 4, 1924)
- Oliver, Wade W, M D Rockefeller Foundation, New York City Associate Director, Division of Medical Sciences (4, 1925)
- Olmsted, J M D, M A, Ph D University of California, Berkeley Professor of Physiology (1, 1920)
- Olsen, Norman S, Ph D Washington Univ Med Sch, St Louis, Mo Asst Professor of Biochemistry (1, 1948)
- Olson, Byron J, Ph D, M D National Institutes of Health, Bethesda, Md Surgeon, Div of Infectious Diseases, USPHS (6, 1948)
- Olson, Carl, Jr, D V M, Ph D Univ of Nebraska, Lincoln, Nebr Chairman, Dept of Animal Pathology and Hygiene (4, 1937)
- Opdyke, David F, Ph D Western Reserve Medical School, Cleveland 6, O Assistant Professor of Physiology (1, 1945)
- Opie, Eugene L, M D, Sc D, LL D Rockefeller Institute for Medical Research, 66th St and York Ave, New York 21, N Y Member, National Academy of Sciences (1, 1906, 4, 1913, 6, 1923)
- Oppenheimer, Enid Tribe 124 E 61st St, New York City Instructor in Physiology, Columbia University (1, 1932)
- Oppenheimer, Ernst, M D Ciba Pharmaceutical Products, Inc, Lafayette Park, Summit, N J Vice-President in charge of Medical Research (3, 1944)
- Oppenheimer Morton Joseph, Ed M, M D 3400 N Broad St, Philadelphia, Pa Professor of Physiology, Temple University School of Medicine (1 1942)
- Orent-Keiles, Elsa, D Sc Bureau of Human Nutrition and Home Economics, U S Department of Agriculture, Beltsville, Md In Charge of Nutrition Investigations, Assistant Chief, Foods and Nutrition Division (2, 1935, 5, 1935)
- Ort, John M, Ph D 401 Codwise Ave, New Brunswick, N J Director of Research, Carroll Dunham Smith Pharmacal Co (2, 1932)
- Orten, Aline Underhill, M S Ph D Wayne Univ College of Medicine, Detroit 26, Mich Research Associate, Dept of Physiological Chemistry (5, 1946)
- Orten, James M, M S Ph D Wayne University College of Medicine Detroit Mich Associate Professor of Physiological Chemistry (2, 1936, 5, 1937)
- Orth, O Sidney, M S, Ph D, M D University of Wisconsin, Service Memorial Institute, Madison, Wis Professor of Pharmacology (1, 1942, 3, 1944)
- Osborne, Stafford L, B P E, M S, Ph D Northwestern University Medical School, 303 E Chicago Ave, Chicago, Ill Professor of Physical Medicine (1, 1941)
- Oser, Bernard L, M S, Ph D Food Research Laboratories, Inc, 48 14 Thirty third St, Long Island City 1, N Y Director (5, 1945)
- Oster, Robert H, Ph D University of Maryland Medical School, Greene and Lombard Sts, Baltimore Assistant Professor of Physiology (1 1938)
- Osterberg, Arnold E, M S, Ph D Medical Dept, Abbott Laboratories, No Chicago, Ill Associate in Medical Dept (2, 1933)
- Osterhout, Marian I Rockefeller Institute for Medical Research, 66th St and York Ave, New York City 21 Associate, Division of General Physiology (1, 1927)
- Osterhout, W J V, Ph D Rockefeller Institute, 66th St and York Ave, New York City Member Emeritus of the Institute, Member of the National Academy of Sciences (1, 1910)
- Otis, Arthur B, A B, Sc M, Ph D Dept of Physiology, Univ of Rochester, Rochester 7, N Y Associate in Physiology (1, 1946)
- Overman, Richard R, B A, M A, Ph D Dept of Physiology, Univ of Tennessee, Memphis, Tenn Assistant Professor (1, 1946)
- Owen, Seward E, M S, Ph D 418 So 20th Ave, Maywood, Ill Major, S E Sn Corps (1, 1938)
- Pace, Donald M, Ph D Dept of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln Associate Professor of Physiology (1, 1944)
- Pace, Nello, Ph D Division of Medical Physics, Donner Laboratory, University of California, Berkeley 4 Research Associate (1, 1947)
- Pack, George T, M D 139 E 36th St, New York City 16 Fellow in Cancer Research, Memorial Hospital (1, 1924)
- Packchianian, Ardzoony, Ph D School of Medicine, University of Texas, Galveston Associate Pro-

- essor of *Bacteriology and Tropical Medicine*, and *Director of Laboratory of Microbiology* (6, 1943)
- Page, Edouard, Ph D Department of Biochemistry, Medical School, Laval University, Quebec, P Q, Canada (1, 1947)
- Page, Ernest W, M D Department of Obstetrics and Gynecology, University of California Hospital, San Francisco 22 *Assistant Professor* (1, 1947)
- Page, Irvine H, M D Cleveland Clinic Foundation, Euclid Ave and 93rd St, Cleveland 6, O *Director of Research* (1, 1937, 2, 1932)
- Painter, Elizabeth E, Ph D University of Illinois School of Medicine, 1853 W Polk St, Chicago *Assistant Professor of Physiology* (1, 1941)
- P'An, S Y, M D College of Physicians and Surgeons, New York City (3, 1941)
- Pangborn, Mary C, Ph D New Scotland Ave, Albany, N Y *Senior Biochemist, New York State Department of Health, Division of Laboratories and Research* (2, 1941)
- Pappenheimer, A M, Jr, Ph D New York Univ College of Medicine, 177 First Ave, New York 16, N Y *Associate Professor of Bacteriology* (2, 1941, 6, 1938)
- Pappenheimer, Alwin M, M D 45 Holden St, Cambridge, Mass *Professor Emeritus of Pathology, Columbia University* (4, 1922)
- Pappenheimer, John R, B S, Ph D Harvard Medical School, Boston, Mass *Associate in Physiology* (1, 1946)
- Park, Edwards A, M D Johns Hopkins Hospital, Baltimore, Md *Emeritus Professor of Pediatrics, Johns Hopkins University* (1 1923)
- Parker, Robert F, M D Lakeside Hospital, 2109 Adelbert Rd, Cleveland, O *Associate Professor of Microbiology* (4, 1942, 6, 1935)
- Parkins, William M, M A, Ph D Route 2, Chariton, Iowa (1, 1939)
- Parpart, Arthur K, Ph D Guyot Hall, Princeton University, Princeton, N J *Professor of Physiology* (1, 1937)
- Parr, Leland W, Ph D The George Washington University School of Medicine, 1335 H St, N W Washington, D C *Professor of Bacteriology* (4, 1940)
- Parrach, Horace O, Ph D Wright-Patterson Air Force Base, Aero Medical Lab, Dayton, Ohio *Res Physiologist* (1, 1948)
- Parsons, Helen T, M D, Ph D University of Wisconsin, Madison *Professor of Home Economics, In Charge of Purnell Research in Nutrition* (2, 1929, 5, 1933)
- Parsons, Robert J, M D Highland Alameda County Hospital, 2701 11th Ave, Oakland, Calif *Pathologist and Director of Laboratories* (4, 1939)
- Paschkis, Karl E, M D 1025 Walnut St, Philadelphia, Pa *Assistant Professor of Medicine, Associate in Physiology, Jefferson Medical College, Chief of Endocrine Clinic, Jefferson Hospital* (1, 1942)
- Patt, Harvey M, Ph D Argonne National Lab, Chicago, Ill *Physiologist* (1, 1948)
- Patterson, Thos L, A M, M S, Ph D, Sc D (hon) Wayne University College of Medicine, 1512 St Antoine St, Detroit, Mich *Research Professor of Physiology* (1, 1920)
- Patterson, Wilbur I, Ph D Federal Security Agency, Food and Drug Administration, Washington, D C *Chief, Organic Analytical Methods* (2, 1948)
- Patton, H D, Ph D, M D Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle 5 *Assistant Professor* (1, 1947)
- Paul, John R, M D, A M 330 Cedar St, New Haven, Conn *Professor of Preventive Medicine, Yale University Medical School* (4, 1927, 6, 1937)
- Pearce, John Musser, M D New York Hospital, 525 East 68th St, New York 21, N Y (4, 1942)
- Pearce, Louise, M D Rockefeller Institute for Medical Research, Princeton, N J *Associate Member in Pathology and Bacteriology* (3, 1915, 1, 1925)
- Pearcy, Frank, Ph D, M D 2606 Oak Lawn Ave, Dallas, Texas (1, 1928)
- Pearlman, William H, Ph D Jefferson Medical College, 1025 Walnut St, Philadelphia 7, Pa *Assistant Professor, Biochemistry Department* (2, 1946)
- Pearse, Herman E, M D School of Medicine and Dentistry, University of Rochester, Crittenden Blvd, Rochester, N Y *Associate Professor of Surgery* (4, 1932)
- Pearson, Harold E, M D, M P H Univ of Southern California Med Sch, Los Angeles, Calif *Assoc Professor of Bacteriology, Medical Microbiologist, Los Angeles County Hospital* (6, 1948)
- Pearson, P B, Ph D A & M College of Texas, College Station *Head of Department of Biochemistry and Nutrition* (2, 1944, 5, 1940)
- Pease, Marshall C, Jr, M D Branchville Rd, R F D 4, Ridgefield, Conn *Historian of American Academy of Pediatrics* (6, 1920)
- Peck, Robert L, Ph D 939 Madison Avenue, Plainfield, N J *Senior Chemist, Merck and Co, Inc* (2, 1947)
- Pemberton, Ralph, M S, M D University of Pennsylvania, Philadelphia *Professor of Medicine, Graduate School of Medicine* (5, 1933)
- Penfield, Wilder G, M D, D Sc McGill University, Montreal, Que, Canada *Professor of Neurology and Neurosurgery* (1, 1932)



- Pennington, Mary Engle, Ph D 233 Broadway, New York 7, N Y *Consultant in Connection with the Handling, Transportation and Storage of Perishables* (2, 1908)
- Penrod, Kenneth E, B S, Ph D Boston Univ School of Medicine, Dept of Physiology, 80 E Concord St, Boston, Mass *Assistant Professor of Physiology* (1, 1946)
- Peoples, S Anderson, M D Baylor University College of Medicine, Houston, Texas *Professor of Pharmacology* (3, 1937)
- Perlman, Ely, A B, M D Mt Sinai Hospital, 100th St and Fifth Ave, New York 29, N Y *Research Associate* (6, 1944)
- Perlzweig, William A, A M, Ph D Box 3711, Duke Hospital, Durham, N C *Professor of Biochemistry, Duke University, Biochemist, Duke Hospital* (2, 1924, 5, 1944)
- Permar, Howard H, M D Pathologic Laboratories, Mercy Hospital, Pittsburgh, Pa *Director of Laboratories* (4, 1925)
- Petermann, Mary L, Ph D Sloan-Kettering Institute, New York City *Associate* (2, 1947)
- Peters, John P, M D 789 Howard Ave, New Haven, Conn *John Slade Ely Prof of Med Yale Univ School of Medicine* (2, 1922)
- Peters, Lawrence, B S, Ph D Dept of Pharmacology, Western Reserve Univ Medical School 2109 Adelbert Rd, Cleveland 6, Ohio *Senior Instructor in Pharmacology* (3, 1946)
- Petersen, William E, M S, Ph D Division of Dairy Husbandry, University of Minnesota, St Paul 1 *Professor* (1, 1947)
- Petersen, William F, M D 1322 Astor St, Chicago *Professor of Pathology, Univ of Illinois* (3, 1923, 4, 1923)
- Peterson, William H, A M, Ph D Biochemistry Building, University of Wisconsin, Madison *Professor of Biochemistry* (2, 1919, 5, 1936)
- Petroff, S A, Ph D, Sc D Route 5, Greenville, South Carolina (6, 1926)
- Pett, L B, M D, Ph D Department of National Health and Welfare, Ottawa, Canada *Director of Nutrition* (2, 1937, 5, 1945)
- Peugnet, Hubert B, M D Department of Surgery, University of Chicago, Chicago, Ill (1, 1938)
- Pfeiffer, Carl C, Ph D, M D Department of Pharmacology, University of Illinois, 1853 West Polk St, Chicago 12 *Professor of Pharmacology and Chairman of Dept* (3, 1938)
- Pfiffner, Joseph J, Ph D Research Laboratories, Parke, Davis & Co, Detroit 32, Mich *Research Chemist* (1, 1931, 2, 1931, 5, 1946)
- Phatak, Nilkanth M, M S, Ph D North Pacific College of Oregon, School of Dentistry, Portland *Associate Professor of Physiology, Pharmacology, and Research and Instructor Dept of Pharmacology, University of Oregon Medical School, Portland Captain, Sn C* (3, 1941)
- Philips, Frederick S, Ph D Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York, N Y *Chief, Pharmacology Department* (3, 1947)
- Phillips, Paul H, Ph D University of Wisconsin, Madison *Professor of Biochemistry* (2, 1940, 5, 1938)
- Phillips, Robert Allan, M D U S Naval Medical Research Unit No 3, American Embassy at Cairo, Egypt, c/o Navy Pouch Section, Navy Department, Washington 25, D C (1, 1938)
- Pick, Ernst Peter, M D 19 E 98th St, New York City *Associate Pharmacologist to the Mt Sinai Hospital, Clinical Professor of Pharmacology in Columbia University* (3, 1940)
- Pierce, Harold B, M S, Ph D College of Medicine, University of Vermont, Burlington *Professor and Chairman, Dept of Biochemistry* (2, 1929, 5, 1933)
- Pierce, Harold Fisher, Ph D, M D State Veterans' Hospital, Rocky Hill, Conn (1, 1928)
- Pierce, Ira H, M S, Ph D Univ of Iowa, Iowa City *Associate Professor of Pharmacology* (3, 1933)
- Pike, Frank H, Ph D 437 W 59th St, New York City 19 *Associate Professor of Physiology, Columbia University* (1, 1907)
- Pillemer, Louis, Ph D Inst of Pathology, Western Reserve Univ, Cleveland, O *Assoc Professor of Immunochimistry* (6, 1942)
- Pincus, Gregory, M S, Sc D Worcester Foundation for Experimental Biology, 222 Maple Ave, Shrewsbury, Mass (1, 1935)
- Pincus, I J, M D Temple Univ Med Sch, Philadelphia, Pa *Res Assoc, Fels Found for Med Res* (1, 1948)
- Pinkerton, Henry, M D St Louis University School of Medicine, St Louis, Mo *Professor of Pathology* (4, 1931)
- Pinkston, James O, Ph D American University of Beirut, Beirut, Lebanon *Pharmacologist* (1, 1936, 3, 1939)
- Pinson, Ernest A, Ph D 139 North Walnut St, Yellow Springs, Ohio (1, 1943)
- Pittman, Martha S, A M, Ph D Manhattan, Kansas (5, 1933)
- Pitts, Robert F, Ph D, M D Syracuse Univ College of Medicine, Syracuse, N Y *Professor of Physiology and Head of the Department of Physiology* (1, 1934)
- Pollack, Herbert, Ph D, M D 45 E 66th St, New York City 21 *Associate Physician and Chief of Metabolism Clinics, Mt Sinai Hospital* (1, 1933, 5, 1935)
- Pomerat, Charles Marc, Ph D University of Texas Medical School, Galveston *Professor of Anatomy* (1, 1944)

- Pommerenke, W T**, A M, Ph D, M D University of Rochester School of Medicine and Dentistry, Rochester 7, N Y *Associate Professor of Obstetrics and Gynecology* (1, 1947)
- Pond, Samuel E**, A M, Ph D 400 S Main St, East Hartford, Conn *Consulting Engineer, P and W A Division, United Aircraft Corp* (1, 1924)
- Ponder, Eric**, M D, Sc D The Nassau Hospital, Mineola, Long Island, N Y (1, 1931)
- Poppen, John R**, M D U S Naval Base Station, Aero-Med Equipment Lab, Philadelphia, Pa *Superintendent* (1, 1948)
- Popper, Hans**, Ph D, M D Cook County Hospital, 1825 W Harrison St, Chicago 12, Ill *Director of Department of Pathology and Scientific Director of Hektoen Institute for Medical Research, Assistant Professor of Pathology, Northwestern University Medical School* (1, 1942)
- Porter, Eugene L**, A M, Ph D University of Texas, Medical Branch, Galveston *Professor of Physiology* (1, 1913)
- Porter, Thelma**, Ph D University of Chicago, Chicago Ill *Prof and Head of Department of Home Economics* (5, 1944)
- Porter, William Townsend**, M D, Sc D, LL D Dover, Mass *Professor Emeritus of Comparative Physiology, Harvard University* (1, 1891)
- Poth, Edgar J**, M D, M A, Ph D Univ of Texas Med School, Galveston, Texas *Professor of Surgery* (1, 1946)
- Potter, Truman S**, M D 82 N Prospect St, Amherst, Mass *Independent Research Worker* (6, 1939)
- Potter, Van R** Ph D McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison *Professor of Oncology* (2, 1941)
- Powell, Horace M**, Sc D 5565 Washington Blvd, Indianapolis, Ind *Head Bacteriologist, Eli Lilly & Co* (6, 1934)
- Power, Marschelle H**, M S, Ph D Mayo Clinic, Rochester, Minn *Professor of Physiological Chemistry, Mayo Foundation, University of Minnesota* (2, 1932)
- Pratt, Joseph H**, A M, M D Sc D New England Medical Center, 25 Bennet St, Boston, Mass *Physician-in-Chief, Boston Dispensary, and Joseph H Pratt Diagnostic Clinic, Professor Emeritus of Clinical Medicine, Tufts Medical School* (1, 1910, 3, 1910, 4, 1927)
- Preisler, Paul W**, M S, Ph D 4580 Scott Ave, St Louis 10, Mo *Assistant Professor of Biochemistry, Washington University School of Medicine* (2, 1931)
- Price, Clifford W**, Ph D U S Food and Drug Administration, Washington, D C *Bacteriologist, Antibiotics Analyst, Div of Penicillin Control and Immunology* (6, 1946)
- Prinzmetal, Myron**, M A, M D Cedars of Lebanon Hospital, Los Angeles, Calif *Senior Attending Physician* (3, 1941)
- Prosser, C Ladd**, Ph D Natural History Building, University of Illinois, Urbana (1, 1935)
- Puestow, Charles B**, M D, M S, Ph D University of Illinois, College of Medicine, 1853 W Polk St, Chicago *Assistant Professor of Surgery* (1, 1934)
- Pugsley, L I**, Ph D Dept Nat'l Health and Welfare Food and Drug Lab, Ottawa, Canada *Chief, Lab Service* (2, 1937)
- Putnam, Frank W**, M A, Ph D University of Chicago, Chicago, Ill *Asst Prof of Biochemistry* (2, 1917)
- Quackenbush, Forrest W**, Ph D R-2, Brookston, Ind *Professor and Head of Dept of Agricultural Chemistry, Purdue Univ* (2, 1946)
- Quaife, Mary L**, Ph D Distillation Products, Inc, Rochester, N Y *Research Chemist* (2, 1948)
- Quastel, J H**, D Sc, Ph D, F R S McGill Univ, Montreal, Canada *Professor of Biochemistry* (2, 1948)
- Queen, Frank B**, M D Univ of Oregon School of Medicine, 3181 S W Marquam Hill Rd, Portland, Oregon *Professor of Pathology* (4, 1941)
- Quick, Armand J**, M D, Ph D 561 N 15th St, Milwaukee 3, Wis *Professor and Director, Dept of Biochemistry, Marquette Medical School*, (2, 1932, 3, 1937)
- Quigley, J P**, M S, Ph D Dept of Pharmacology, Univ of Tenn, Memphis 3, Tenn *Professor and Chief of the Division of Pharmacology* (1, 1929, 3, 1945)
- Quinn, Edmond John**, Ph D 106 N Lee Ave, Rockville Center, Long Island, N Y *Medicinal Sales Division, Merck & Co, Inc, Rahway, N J* (2, 1927, 5, 1933)
- Rabinowitch, I M**, O B E, D Sc, M D, C M, F R C P, (c), F A C P 1020 Medical Arts Building, Sherbrooke and Guy Streets, Montreal, Canada *Associate Professor of Medicine and Lecturer in Medical Jurisprudence and Toxicology, McGill University, Director, Institute for Research, Montreal General Hospital* (2, 1928, 5, 1933)
- Rachele, Julian R**, Ph D Cornell Univ Medical College, New York City *Asst Professor of Biochemistry* (2, 1948)
- Rackemann, Francis M**, M D 263 Beacon St, Boston, Mass *Physician, Massachusetts General Hospital, Lecturer in Medicine, Harvard Medical School* (6, 1923)
- Racker, Efraim**, M D New York Univ College of Medicine, New York City *Asst Professor of Microbiology* (2, 1948)
- Raffel, Sidney**, Sc D, M D Department of Bacteriology and Experimental Pathology, Stan-

- ford University, Calif Assistant Professor (6, 1938)
- Rahn, Hermann, Ph D University of Rochester School of Medicine and Dentistry, Rochester, N Y Assistant Professor of Physiology (1, 1944)
- Rake, Geoffrey W, M B, M R C S, L R C P Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N J Head, Division of Microbiology (6, 1939)
- Rakestraw, Norris W, Ph D Scripps Institution of Oceanography, University of California, LaJolla Professor of Chemistry (2, 1925)
- Rakieten, Nathan, Ph D Bristol Laboratories, Inc, P O Box 657, Syracuse 2, N Y Pharmacologist and Toxicologist (1, 1941)
- Ralli, Elaine P, M D 477 First Ave, New York City Associate Professor of Medicine, New York University College of Medicine (1, 1934, 5, 1933)
- Ralston, H J, Ph D Department of Physiology, College of Physicians and Surgeons, 344 14th St, San Francisco 3, Calif Assistant Professor of Physiology (1, 1947)
- Rammelkamp, Charles H, Jr, B A, M D Dept of Preventive Medicine, Western Reserve Univ, Cleveland 6, Ohio Assoc Professor of Medicine (6, 1943)
- Ramsey, Robert Weberg, M S, Ph D Medical College of Virginia, Richmond Associate Professor of Physiology and Pharmacology (1, 1939)
- Randall, Lowell O, Ph D Hoffmann LaRoche, Inc, Nutley 10, N J Pharmacologist (2, 1939)
- Randall, Walter C, M S, Ph D St Louis University, School of Medicine, 1402 S Grand Blvd, St Louis, Mo Instructor in Physiology (1, 1943)
- Randall, William A, B S, M S, Ph D Food and Drug Administration, Washington 25, D C Bacteriologist, Division of Penicillin Control and Immunology (6, 1946)
- Rane, Leo, Ph D Lederle Laboratories, Inc, Pearl River, N Y Department Head, Normal Blood Plasma (6, 1942)
- Rantz, Lowell A, A B, M D Stanford Univ Hospital, San Francisco 15, Calif Assistant Professor of Medicine (3, 1946)
- Rapoport, Samuel, M D, Ph D The Children's Hospital Research Foundation, Elland and Bethesda, Cincinnati, O Research Associate (2, 1941)
- Rapport, David, M D 416 Huntington Ave, Boston, Mass Professor of Physiology, Tufts College Medical School (1, 1922)
- Raska, Sigwin, Ph D, M A University of Mississippi, School of Medicine, University, Miss Professor of Pharmacology (1, 1947)
- Rasmussen, Andrew Theodore, Ph D University of Minnesota Medical School, Minneapolis Professor of Neurology (1, 1919)
- Ratner, Bret, M D 50 E 78th St, New York City Professor of Pediatrics, New York Univ College of Medicine (4, 1940, 6, 1928)
- Ratner, Sarah, Ph D Dept of Pharmacology, N Y Univ College of Medicine, 477 First Ave, New York 16, N Y Assistant Professor of Pharmacology (2, 1944)
- Raulston, B O, A B, M D 200 S Hudson Ave, Los Angeles, Calif Professor of Medicine, Director of Clinical Teaching, and Associate Dean, the University of Southern California, School of Medicine (3, 1942)
- Ravdin, I S, M D University of Pennsylvania School of Medicine, Philadelphia John Rhea Barton Professor of Surgery, Chief Surgeon, Hospital of the University of Pennsylvania (1, 1930, 4, 1930)
- Rawson, Rulon W, M D Memorial Hospital, New York City Attending Physician, Chief, Dept of Clinical Investigation, Sloan-Kettering Institute, Associate Professor of Medicine, Cornell Medical College (1, 1947)
- Raymond, Albert L, Ph D G D Searle & Co, P O Box 5110, Chicago 80, Ill Vice President (2, 1932)
- Reback, John F, B S, M S 615 N Wolfe St, Baltimore, Md Student School of Hygiene, Johns Hopkins University (6, 1943)
- Redfield, Alfred C, Ph D Woods Hole, Mass Professor of Physiology, Harvard University (1, 1919)
- Reed, Carlos Isaac, A M, Ph D College of Medicine, University of Illinois, 1853 W Polk St, Chicago Professor of Physiology (1, 1923)
- Reed, Emerson A, Ph D Hahnemann Medical Col, Philadelphia, Pa Asst Professor of Physiology (1, 1948)
- Reed, Howard S, Ph D 3048 Life Sciences Bldg, University of California, Berkeley Professor of Plant Physiology (2, 1909)
- Rehm, Warren S, Jr, Ph D, M D University of Louisville School of Medicine, Louisville, Ky Assistant Professor of Physiology (1, 1945)
- Reid, Mary E, Ph D National Institute of Health, Bethesda 14, Md Cytologist (5, 1947)
- Reid, Marion Adelaide, A M, Ph D New Jersey College for Women, New Brunswick, N J (1, 1941)
- Reimann, Hobart A, M D Jefferson Hospital, Philadelphia, Pa Professor of Medicine, Jefferson Medical College (4, 1933)
- Reimann, Stanley P, M D, Sc D 703 W Philadelphia St, Mount Airy, Philadelphia, Pa Director of the Research Institute of the Lankenau Hospital, Director, Institute of Cancer Research Associate Professor of Surgical Pathology,

- Graduate School of Medicine, University of Pennsylvania, Professor of Oncology, Hahnemann Medical College and Hospital, Philadelphia* (1, 1921, 4, 1924)
- Reinecke, Roger M**, M A, M B, Ph D, M D Department of Physiology, University of Minnesota, Minneapolis 14 *Assistant Professor* (1, 1947)
- Reiner, John M**, M S, Ph D Tufts College Medical School, Dept of Physiology, Boston, Mass (1, 1947)
- Reiner, Laszlo**, M D, Ph D 165 Franklin St, Bloomfield, N J *Director, Pharmaceutical Research, Wallace & Trieman Products, Inc* (2, 1942, 6, 1933)
- Reinhold, John G**, M S, Ph D University of Pennsylvania Hospital, Pepper Lab, Philadelphia, Pa *Associate in charge of Biochemistry* (2, 1936)
- Remington, John W**, M S, Ph D University of Georgia, School of Medicine, Augusta *Assistant Professor of Physiology* (1, 1943)
- Remington, Roe E**, M A, Ph D, D Sc Hendersonville, N C *Consultant* (2, 1930, 5, 1934)
- Renfrew, Alice G**, Ph D Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa *Senior Fellow* (2, 1939)
- Renshaw, Birdsey**, M A, Ph D University of Oregon Medical School, Portland (1, 1941)
- Reynolds, Chapman**, M D Louisiana State University, School of Medicine, New Orleans *Assistant Professor of Pharmacology* (3, 1937)
- Reynolds, Orr E**, Ph D Office of Naval Research, Med Sciences Div, Washington, D C *Head, Physiology Branch* (1, 1948)
- Reynolds, Samuel R M**, Ph D 4028 Deepwood Rd, Baltimore 18, Md *Carnegie Institution of Washington, Dept of Embryology, Staff Member and Physiologist* (1, 1932)
- Reznikoff, Paul**, M D New York Hospital, 525 E 68th St, New York City *Associate Professor of Clinical Medicine, Cornell University Medical College* (1, 1927)
- Rhoads, Cornelius Packard**, M D Memorial Hospital, 444 E 68th St, New York City *Director, Professor of Pathology, Cornell University Medical College, Director of Sloan-Kettering Institute for Cancer Research* (4, 1930)
- Rhoads, Jonathan Evans**, B A, M D, D Sc 4023 Pine St, Philadelphia 4, Pa *Assistant Professor of Surgical Research* (1, 1946)
- Rice, Christine E**, Ph D Animal Diseases Research Inst, Canadian Dept of Agriculture, Hull, Quebec, Canada *Agricultural Scientist* (6, 1938)
- Rice, Harold V**, Ph D Univ of Manitoba, Winnipeg, Manitoba, Canada *Professor of Physiology* (1, 1948)
- Rice, James C**, A M, Ph D University of Mississippi, P O Box 173, University *Professor of Pharmacology* (3, 1911)
- Rich, Arnold Rice**, M D Johns Hopkins Hospital, Baltimore, Md *Bazley Professor of Pathology, Johns Hopkins University* (4, 1924)
- Richards, Alfred N**, Ph D, Sc D, M D (hon), LL D Univ of Pennsylvania School of Medicine, Philadelphia *Emeritus Professor of Pharmacology, Chairman, National Academy of Sciences* (1R, 1900, 2, 1906, 3R, 1909)
- Richards, Oscar W**, M A, Ph D American Optical Co, Scientific Instrument Division, Box A Buffalo 15, N Y *Chief Biologist* (1, 1934)
- Richards, Richard Kohn**, M D Abbott Laboratories, North Chicago, Ill *Director, Pharmacologic Research, Lecturer in Pharmacology, Northwestern University Medical School, Chicago* (1, 1938, 3, 1947)
- Richardson, Arthur P**, M D Department of Pharmacology, Emory University School of Medicine, Emory University, Ga *Professor of Pharmacology* (3, 1939)
- Richardson, Luther R**, Ph D P O Box 102, College Station, Texas (5, 1942)
- Richter, Curt P**, Ph D Phipps Psychiatric Clinic, The Johns Hopkins Hospital, Baltimore, Md *Associate Professor of Psycho-biology, Johns Hopkins University* (1, 1924)
- Richter, Maurice N**, M D 303 E 20th St, New York City *Professor of Pathology, New York University Medical School, Director, Department of Pathology, New York Post-Graduate Hospital* (4, 1931)
- Ricketts, Henry T**, M D Dept of Medicine, University of Chicago, Chicago, Ill *Associate Professor of Medicine* (1, 1940)
- Riegel, Byron**, A M, Ph D Department of Chemistry, Northwestern University, Evanston, Ill *Professor* (2, 1942)
- Riegel, Cecilia**, M S, Ph D Room 563, University Hospital, Philadelphia, Pa *Research Associate, Department of Research Surgery, University of Pennsylvania School of Medicine* (2, 1938)
- Ries, Fred A**, M D 139 East North Ave, Baltimore 2, Md *Instructor in Neurology, Johns Hopkins University* (1, 1933)
- Rigdon, R H**, M D Medical Branch, Univ of Texas, Galveston *Professor of Experimental Pathology* (4, 1941)
- Riggs, Douglas S**, M D Harvard Medical School, Boston, Mass *Instructor in Pharmacology* (3, 1948)
- Riggs, Lloyd K**, Ph D National Dairy Research

- Laboratories, Oakdale, L I , N Y *Director, Nutritional Res* (2, 1929)
- Riker, Walter F , Jr , M D Cornell University Medical College, 1300 York Avenue, New York, 21, N Y *Assistant Professor of Pharmacology* (3, 1947)
- Riley, Richard L , M D Columbia Univ , New York City *Asso in Medicine* (1, 1948)
- Rinehart, James F , M D University of California Medical School, Parnassus and Third Aves , San Francisco *Professor of Pathology* (4, 1933)
- Ring, Gordon C , M A , Ph D Temple University Medical School, Broad St , Philadelphia, Pa (1, 1933)
- Rioch, David McKenzie, M D Chestnut Lodge Sanitarium, 500 W Montgomery Ave , Rockville, Md *Director of Research* (1, 1931)
- Rittenberg, David, Ph D 630 W 168th St , New York City *Associate Professor, College of Physicians and Surgeons, Columbia University* (2, 1939)
- Ritzman, E G , A M , Science (hon ) University of New Hampshire, Durham *Research Professor* (5, 1933)
- Rivers, T M , M D , Sc D The Hospital of the Rockefeller Institute for Medical Research, 66th St and York Ave , New York City *Director of the Hospital, Member of the National Academy of Sciences* (4, 1925, 6, 1921)
- Robb, Jane Sands, Sc D , M D College of Medicine, Syracuse University, 761 Irving Ave , Syracuse, N Y *Associate Professor of Pharmacology* (1, 1924)
- Robbie, W A , M S , Ph D Department of Ophthalmology, College of Medicine, State University of Iowa, Iowa City *Research Associate Professor of Ophthalmology and Physiology* (1, 1947)
- Robbins, Benjamin Howard, M S , M D Vanderbilt Univ School of Medicine, Nashville, Tenn *Associate Professor of Pharmacology* (3, 1936)
- Robbins, Mary L , B A , M A , Ph D George Washington Univ School of Medicine, 1335 H Street, N W , Washington 5, D C *Asst Professor of Bacteriology* (6, 1946)
- Roberts, Edward F , M D , Ph D Wyeth, Inc 1600 Arch St , Philadelphia 3, Pa *Director of Clinical Investigation* (6, 1932)
- Roberts, Joseph T , M S , M D , Ph D Veterans Administration, Buffalo, N Y *Chief of Medical Service* (1, 1947)
- Roberts, Lydia J , Ph D University of Chicago, Chicago, Ill *Professor and Chairman of Department of Home Economics* (5, 1933)
- Roberts, Sidney, S B , M S , Ph D University of California, Medical School, Los Angeles *Assistant Clinical Professor of Physiological Chemistry* (1, 1946)
- Robertson, Elizabeth Chant, M D , M A , Ph D University of Toronto, Toronto, Canada *Research Fellow in Paediatrics* (5, 1939)
- Robertson, Oswald H , M D University of Chicago, Chicago, Ill *Professor of Medicine* (4, 1932)
- Robinson, Charles Summers, Ph D Medical School, Vanderbilt University, Nashville, Tenn *Professor of Biochemistry* (2, 1925)
- Robinson, Elliott S , B A , M D , Ph D R F D #4, Laconia, N H *Director, Division of Biologic Laboratories, Mass Dept of Health (Leave of Absence)* (6, 1935)
- Robinson, G Canby, M D , Sc D , LL D Johns Hopkins Hospital, Baltimore, Md *Lecturer in Medicine, Johns Hopkins University* (1R, 1912, 3, 1921)
- Robinson, Harry J , Ph D Merck Institute for Therapeutic Research, Rahway, N J *Assistant Director* (3, 1946)
- Robinson, Herbert E , Ph D Swift and Company, Research Laboratories, Union Stock Yards, Chicago 9, Ill *Assistant Director of Research* (5, 1947)
- Robinson, Howard W , M S , Ph D Broad and Ontario Sts , Philadelphia, Pa *Professor of Physiological Chemistry, Temple University School of Medicine* (2, 1929)
- Robinson, Sid, Ph D Indiana University Medical School, Bloomington *Professor of Physiology* (1, 1941)
- Robinson, True W , Ph D Wright-Patterson Air Force Base, Aero Medical Lab , Dayton, Ohio *Chief, Metabolism Unit* (1, 1948)
- Roblin, Richard O , Jr , M A , Ph D 1937 West Main St , Stamford, Conn *Director, Chemotherapy Division, American Cyanamid Co* (2, 1946, 6, 1947)
- Robschert-Robbins, F S , Ph D University of Rochester School of Medicine and Dentistry, Rochester, N Y *Associate in Pathology* (1, 1925, 4, 1930)
- Rodbard, Simon, Ph D Cardiovascular Dept , Michael Reese Hospital, 29th and Ellis Aves , Chicago, Ill (1, 1942)
- Roe, Joseph Hiram, M A , Ph D George Washington University School of Medicine, Washington, D C *Professor of Biochemistry* (2, 1927, 5, 1933)
- Roeder, Kenneth D , M A Tufts College, Medford, Mass *Associate Professor of Biology* (1, 1942)
- Roepke, Martin Henry, Ph D University Farm, St Paul, Minn *Professor, Veterinary Medicine* (3, 1937)
- Rogers, Fred T , A M , Ph D , M D Dallas Medical and Surgical Clinic, 4105 Live Oak St , Dallas 1, Texas (1, 1917)
- Rogoff, Julius M , Ph G , M D , Sc D School of

- Medicine, University of Pittsburgh, Pittsburgh, Pa *Professor of Endocrinology* (1, 1916, 3, 1916)
- Ronzoni, Ethel, M A, Ph D Washington University Medical School, St Louis 4, Mo *Assistant Professor of Biological Chemistry* (2, 1923)
- Root, Howard F, M D 44 Dwight St, Brookline, Mass *Associate in Medicine, Harvard Medical School* (5, 1933)
- Root, Walter S, Ph D College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York City *Professor of Physiology* (1, 1932)
- Rosahn, Paul D, M D 92 Grand St, New Britain, Conn *Pathologist, New Britain General Hospital, Associate Clinical Professor of Pathology, Yale University School of Medicine, New Haven* (4, 1934)
- Rose, William C, Ph D, D Sc University of Illinois, Urbana *Professor of Biochemistry, and Acting Head of Chemistry Dept, Member, National Academy of Sciences* (2, 1912, 5, 1933)
- Rosenblueth, Arturo, M D Instituto Nacional de Cardiologia, Calzada de la Piedad 300, Mexico D F, Mexico (1, 1932)
- Rosenfeld, Morris, M D Johns Hopkins School of Medicine, Baltimore, Md *Associate in Pharmacology and Experimental Therapeutics Captain, M C, U S A* (3, 1934)
- Rosenow, Edward C, M D, hon LL D and D Sc Research Dept, Longview State Hospital, Cincinnati 16, Ohio (4, 1913, 6, 1915)
- Rosenthal, Otto, M D University of Pennsylvania School of Medicine, Philadelphia, Pa *Asst Prof of Cancer Research, Harrison Dept of Surgical Research* (2, 1946)
- Rosenthal, Sanford M, M D National Institute of Health, Bethesda, Md *Senior Pharmacologist, U S Public Health Service* (3, 1925)
- Rosenthal, S R, M D, Ph D University of Illinois College of Medicine, Chicago *Assistant Professor of Bacteriology and Public Health, Director, Tice Laboratory for B C G Vaccination against Tuberculosis, Municipal Tuberculosis Sanatorium* (1, 1948, 4, 1941)
- Ross, Joseph F, M D The Robert Dawson Evans Memorial, 65 E Newton St, Boston, Mass *Member of the Department, Physician, Massachusetts Memorial Hospital, Associate Professor of Medicine, Boston University School of Medicine* (4, 1941)
- Rossiter, R J, Ph D, F R I C Univ of Western Ontario, London, Ontario, Canada *Professor of Biochemistry* (2, 1948)
- Rostorfer, Howard Hayes, B A, M S, Ph D Department of Physiology, Indiana University, Bloomington, Indiana *Assistant Professor of Physiology* (1, 1946)
- Roth, Grace M, M S, Ph D Mayo Clinic, Rochester, Minn *Associate in Clinical Physiology* (1, 1939)
- Roth, L W, M A, Ph D Department of Pharmacology, The Abbott Research Laboratories, North Chicago, Ill *Research Pharmacologist* (1, 1947)
- Rothmund, Paul W K, Dipl-Ing, Dr-Ing (Munich) Antioch College, Yellow Springs, O *Professor of Chemistry and Research Chemist, The C F Kettering Foundation, Associate Professor, Department of Chemistry, Ohio State University* (2, 1940)
- Rous, Peyton, M D, Sc D Rockefeller Institute for Medical Research, York Ave at 66th St, New York City *Member, Member of the National Academy of Sciences* (4, 1913)
- Routh, Joseph I, M S, Ph D State University of Iowa, Iowa City *Associate Professor of Biochemistry* (2, 1942)
- Rovenstine, Emery Andrew, A B, M D 477 First Ave, New York, N Y *Professor of Anesthesia, New York University, Director, Division of Anesthesia, Bellevue Hospital* (3, 1944)
- Rowntree, Jennie I, M S, Ph D University of Washington, Seattle *Professor of Home Economics* (5, 1933)
- Rowntree, L G, M D, Sc D, F A C P DuPont Building, E Flagler St at 2nd Ave, Miami, Fla (1, 1911, 2, 1910, 3, 1908, 4, prior to 1920, 5, 1933)
- Rubenstein, Boris B, Ph D, M D Dept of Metabolic & Endocrine Research, Michael Reese Hospital, East 59th St & Ellis Ave, Chicago, Ill (1, 1934)
- Rubin, Saul H, M S, Ph D Hoffmann La Roche, Inc, Nutley 10, N J *Director, Nutrition Laboratories* (2, 5, 1947)
- Ruch, Theodore C, M A, Ph D 204B Physiology Hall, University of Washington, Seattle 5 *Professor of Physiology and Biophysics* (1, 1933)
- Ruchman, Isaac, Ph D Cincinnati General Hospital, Cincinnati, Ohio *Asst Professor of Bacteriology* (6, 1948)
- Rusch, Harold Paul, M D University of Wisconsin Medical School, Mc Ardle Memorial Laboratory, Madison 6 *Professor of Oncology Director of Mc Ardle Memorial Laboratory for Cancer Research* (4, 1940)
- Rusoff, Louis L, Ph D Louisiana State Univ, Baton Rouge, La *Assoc Dairy Nutritionist in Experiment Station and Assoc Professor of Nutrition* (5, 1948)
- Russell, Walter C, Ph D, Sc D (hon) New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick *Research Specialist and Professor of Agricultural Biochemistry* (2, 1932, 5, 1933)
- Ryan, Andrew Howard, M D Chicago Medical School, 710 S Wolcott Ave, Chicago, Ill *Asso-*

- ciate Professor of Physiology and Pharmacology* (1, 1912)
- Briland, David A**, M D *Stanford Univ Hospital, San Francisco 15, Calif Associate Professor of Medicine, Stanford Univ School of Medicine* (3, 1946)
- Babin, Albert**, A S, M D *Children's Hospital Research Foundation, Cincinnati, Ohio Professor of Research Pediatrics, Univ of Cincinnati* (6, 1946)
- Backs, Jacob**, Ph D, M D *Biology Dept, Brookhaven National Laboratory, Upton, L I, N Y Scientist* (1, 1948, 3, 1933)
- Bah, Peter P T**, M S, Ph D *Division of Pharmacology and Experimental Therapeutics, Univ of Calif, San Francisco, Calif Lecturer in Pharmacology* (3, 1941)
- Bahyun, Melville**, A M, Ph D *Frederick Stearns and Company Division, Sterling Drug, Inc, Detroit, Mich Vice President and Director of Research* (2, 1932)
- Balk, Jonas E**, M D *School of Medicine, University of Pittsburgh, Pittsburgh, Penn Associate Research Professor of Bacteriology* (6, 1947)
- Salmon, W D**, A M *Alabama Polytechnic Institute, Auburn Animal Nutritionist* (2, 1929, 5, 1933)
- Salter, William T**, B A, M D *Yale School of Medicine, 333 Cedar St, New Haven, Conn Professor of Pharmacology* (1, 1933, 3, 1942, 5, 1934)
- Sammis, Florence E**, M D *133 E 58th St, New York City Physician, Allergy, O P D, New York Hospital* (6, 1943)
- Sampson, John J**, M D *490 Post St, San Francisco, Calif* (1, 1932)
- Sampson, Myra M**, A M, Ph D *Smith College, Northampton, Mass Professor of Zoology* (5, 1935)
- Samuels, Leo T**, Ph D *University of Utah Medical School, Salt Lake City Professor and Head of Dept of Biochemistry* (2, 1941, 3, 1937)
- Sandels, Margaret R**, A M, Ph D *Florida State University, Tallahassee Dean of School of Home Economics, Professor of Nutrition* (5, 1933)
- Sandiford, Irene**, Ph D *Billings Hospital, University of Chicago, Chicago, Ill Assistant Professor of Medicine* (2, 1925, 5, 1933)
- Sandow, Alexander**, Ph D *Washington Square College, New York University, New York 3, N Y Assistant Professor of Biology* (1, 1945)
- Sandweiss, David J**, M D *9739 Dexter Ave, Detroit, Mich Instructor in Clinical Medicine, Wayne University College of Medicine, Physician, Harper Hospital (OPD), Attending Physi-*
- cian Gastroenterology and Gastroscopy, North End Community Fund Clinic* (1, 1944)
- Sanford, Arthur H**, A M, M D *Clinical Laboratories, Mayo Clinic, Rochester, Minn Head, Division of Clinical Laboratories* (6, 1920)
- Santos, Francisco O**, M S, Ph D *University of the Philippines, Los Banos, Laguna Professor and Head of Department of Agricultural Chemistry, College of Agriculture* (5, 1936)
- Saphur, Otto**, M D *Michael Reese Hospital, 29th St and Ellis Ave, Chicago 16, Ill Pathologist, Michael Reese Hospital, Clinical Professor of Pathology, University of Illinois Medical School* (4, 1927)
- Saphra, Ivan**, M D *Beth Israel Hospital, New York City Assoc Bacteriologist* (6, 1946)
- Sarett, Herbert P**, M S, Ph D *Tulane Medical School, 1430 Tulane Ave, New Orleans 13, La Assistant Professor of Biochemistry* (2, 1946, 5, 1947)
- Saslow, George**, Ph D, M D *Department of Neuropsychiatry, Washington University Medical School, 640 South Kingshighway, St Louis, Mo Assistant Professor of Psychiatry Associate Physician to the Student Health Service* (1, 1936)
- Satterfield, G Howard**, A M *State College of Agriculture and Engineering, University of North Carolina, Raleigh Professor of Biochemistry* (2, 1944, 5, 1941)
- Saul, Leon Joseph**, M A, M D *Room 1907, 255 S 17th St, Philadelphia 3, Pa* (1, 1933)
- Saunders, Felix**, Ph D *231 Playa del Sur, La Jolla, Calif* (2, 1938)
- Sawyer, Margaret E MacKay**, M A, Ph D *142 Lower Albert St, Kingston, Ontario, Canada* (1, 1935)
- Sawyer, Wilbur A**, M D *3927 Idaho Ave, N W, Washington, D C* (4, 1930)
- Saxton, John A, Jr**, M D *Snodgrass Laboratory of Pathology and Bacteriology, 1430 Carroll St, St Louis, Mo Assistant Professor of Pathology, Washington University School of Medicine, Laboratory Director, Hospital Division, City of St Louis* (4, 1944)
- Sayers, George**, M S, Ph D *Department of Pharmacology, University of Utah, Salt Lake City 1, Utah Assistant Professor of Pharmacology* (1, 1948, 3, 1947)
- Scammon, Richard E**, M A, Ph D *172 S E Bedford St, Minneapolis, Minn Distinguished Service Professor in the Graduate School, University of Minnesota* (1, 1923)
- Scantlebury, Ronald E**, Ph D *Stanford Univ Res Inst, Stanford, Calif Director, Dept of Physiology* (1, 1948)
- Schales, Otto**, D Sc *Ochsner Clinic, Prytanis and Aline Sts, New Orleans, La Director of Chemical Research, Ochsner Foundation, Director of the*

- Biochemical Laboratory, Ochsner Clinic, Assistant Professor of Biochemistry, Tulane University School of Medicine* (2, 1944)
- Scharles, Frederick H**, M D 911 Alma Ave, Oakland, Calif (5, 1935)
- Schattenberg, Herbert John**, M S, M D Laboratory of Clinical Pathology, 220-222 Medical Arts Bldg, San Antonio, Texas *Director* (4, 1940)
- Schenken, John R**, M D Univ of Nebraska College of Medicine, Omaha, Neb *Professor of Pathology and Bacteriology* (4, 1942)
- Scherago, Morris**, D V M Univ of Kentucky, Lexington, Ky *Professor and Head, Dept of Bacteriology* (6, 1948)
- Scherp, Henry W**, M S, Ph D Univ of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester 7, N Y *Associate Professor of Bacteriology and Immunology* (6, 1940)
- Schick, Bela**, M D 17 E 84th St, New York City *Pediatrician, Mt Sinai Hospital, Sea View Hospital* (6, 1924)
- Schiffman, Milton J**, M S, Ph D Hoffmann-La Roche, Inc, Chicago, Ill *Research Consultant* (1, 1943)
- Schiller, Alfred A**, M D Univ of Illinois College of Med, Chicago, Ill *Asst Professor of Physiology* (1, 1948)
- Schlenk, Fritz**, Ph D Science Hall, Iowa State College, Ames *Professor of Bacteriology* (2, 1942)
- Schlesinger, M J**, Ph D, M D Beth Israel Hospital, 330 Brookline Ave, Boston, Mass *Assistant Professor of Pathology, Harvard Medical School, Director of Pathology, Beth Israel Hospital* (4, 1942, 6, 1921)
- Schlomovitz, Benjamin H**, M D 1210 Majestic Bldg, 231 W Wisconsin Ave, Milwaukee, Wis *Director, Clinical and Research Laboratory, Veterans Administration Hospital, Wood, Wisconsin* (1, 1919)
- Schlumberger, Hans G**, M D Ohio State University School of Medicine, Columbus *Associate Professor in Pathology* (4, 1945)
- Schmeisser, Harry C**, M D University of Tennessee, Memphis *Professor of Pathology* (4, 1937)
- Schmidt, Carl F**, M D Medical School, University of Pennsylvania, Philadelphia *Professor of Pharmacology* (1, 1929, 3, 1924)
- Schmidt, C Robert**, Ph D, M D Hertzler Clinic, Halstead, Kan *Resident Surgeon Major (MC) A U S* (1, 1940)
- Schmidt, Gerhard**, M D Boston Dispensary, 25 Bennett St, Boston, Mass *Senior Research Fellow, Tufts College Medical School* (2, 1939)
- Schmidt, L H**, Ph D Christ Hospital Institute of Medical Research, Cincinnati, O *Director of Research, Associate Research Professor of Biochemistry, College of Medicine, University of Cincinnati* (2, 1936, 3, 1946)
- Schmitt, Francis Otto**, Ph D Dept of Biology, Massachusetts Institute of Technology, Cambridge *Professor of Biology* (1, 1930)
- Schmitt, Otto H**, Ph D Department of Physics, University of Minnesota, Minneapolis 14 *Associate Professor of Zoology and Physics* (1, 1917)
- Schnedorf, Jerome G**, M D, Ph D 3111 Argonne Circle, Santa Barbara, Calif (1, 1941)
- Schneider, Edward C**, Ph D, Sc D, 25 Gordon Place, Middletown, Conn *Professor of Biology (retired), Wesleyan University* (1R, 1912, 2, 1912)
- Schneider, Howard A**, M S, Ph D The Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N Y *Associate* (5, 1947)
- Schneerson, S Stanley**, M D Mount Sinai Hospital, 2 East 100th St, New York 29, N Y *Associate Bacteriologist* (6, 1946)
- Schoenbach, Emanuel B**, M D Johns Hopkins School of Hygiene, 615 N Wolfe St, Baltimore, Md *Associate Professor of Preventive Medicine* (6, 1941)
- Schoepfle, Gordon M**, A M, Ph D Washington University, School of Medicine, St Louis, Mo *Assistant Professor of Physiology* (1, 1943)
- Scholander, P F**, M D, Ph D Department of Zoology, Swarthmore College, Swarthmore, Pa *Research Biologist* (1, 1947)
- Schradieck, Constant E**, M D General Delivery Germantown, Philadelphia, Pa (6, 1921)
- Schreiner, Oswald**, M S, Ph D Bureau of Plant Industry, U S Department of Agriculture, Washington 25, D C *Chief, Division of Soil Fertility Investigations* (2, 1908)
- Schroeder, E F**, M S, Ph D G D Searle & Co, P O Box 5110, Chicago 80, Ill *Research Biochemist* (2, 1938)
- Schroeder, Henry A**, M D Hypertension Division, Department of Internal Medicine, Washington University, 640 S Kingshighway, St Louis 10, Mo (1, 1947)
- Schubert, Maxwell**, A M, Ph D Department of Therapeutics, N Y U College of Medicine, 477 First Avenue, New York 10, N Y *Research Associate in Therapeutics* (3, 1947)
- Schuck, Cecelia**, Ph D Purdue University, Lafayette, Ind *Professor of Nutrition, Department of Home Economics* (5, 1941)
- Schultz, Edwin William**, M D 743 Cooksey Lane, Stanford University, Calif *Professor of Bacteriology and Experimental Pathology* (4, 1927, 6, 1928)
- Schultz, Fred H, Jr**, Ph D Commercial Sol-



- vents Corporation, Terre Haute, Ind *Research Pharmacologist* (3, 1948)
- Schultze, Max O , Ph D Division of Agricultural Biochemistry, Univ of Minnesota, St Paul 8, Minn *Professor* (2, 1938)
- Schweizer, Malvina, Ph D Washington Square College of Arts and Sciences, New York University, New York, N Y *Instructor in Biology* (1, 1944)
- Schwerma, Henry, Ph D Northwestern Univ , Chicago, Ill *Instr in Physiology* (1, 1948)
- Schwimmer, Sigmund, M S , Ph D Enzyme Research Laboratory, 800 Buchanan Street, Albany 6, Calif *Chemist*, U S Department of Agriculture, U S Bureau of Agricultural and Industrial Chemistry (2, 1947)
- Scott, Charles Covert, Ph D , M D Inlow Clinic, Shelbyville, Ind (3, 1945)
- Scott, David Alymer, M A , Ph D Connaught Laboratories, University of Toronto, Toronto 5, Ontario, Canada *Research Member* (2, 1935)
- Scott, Ernest L , Ph D 64 South St , Bogota, N J *Associate Professor of Physiology, Emeritus, Columbia University* (1R, 1914, 2, 1915)
- Scott, Frederick Hughes, Ph D , Sc D , M B University of Minnesota, Minneapolis *Professor of Physiology, Emeritus* (1R, 1908, 2, 1909)
- Scott, John C , Ph D Hahnemann Medical College, Philadelphia, Pa *Professor of Physiology and Head of the Department* (1, 1936)
- Scott, R W , A M , M D City Hospital, Cleveland, O *Professor of Clinical Medicine, Western Reserve University, Physician-in-Chief, Cleveland City Hospital* (1, 1917, 3, 1917)
- Scott, V Brown, Ph D , M D Inlow Clinic, Shelbyville, Ind *Internist, Division of Medicine* (1, 1941)
- Scott, W J Merle, M D University of Rochester Medical School, Rochester, N Y *Associate Professor of Surgery* (4, 1925)
- Scott, W W , Ph D , M D Brady Urological Inst , The Johns Hopkins Hospital, Baltimore 5, Md (1, 1943)
- Scudi, John V Ph D Pyridium Corporation, Nepera Park, Yonkers 2, N Y *Director of Research* (2, 1942, 5, 1945)
- Seager, Lloyd D , M S , M D Woman's Medical College of Pennsylvania, East Falls, Philadelphia *Professor of Pharmacology and Toxicology* (3, 1939)
- Sealock, Robert R , Ph D Iowa State College, Ames *Associate Professor of Chemistry* (2, 1940, 5, 1941)
- Seastone, C V , Jr , M D University of Wisconsin Medical School, Madison *Professor of Medical Bacteriology, Dept Chairman* (6, 1939)
- Sebrell, W H , Jr , M D National Institutes of Health, Bethesda, Md *Director, Experimental Biology and Medicine Institute, Professorial Lecturer on Nutrition, George Washington University* (2, 1938, 5, 1937)
- Seecof, David P , M D 1970 Daly Ave , Bronx, New York City (4, 1927)
- Seegal, David, M D Maimonides Hospital, Brooklyn, N Y *Director of Medical Services* (6, 1930)
- Seegers, Walter H , Ph D Wayne University College of Medicine, Detroit 26, Mich *Professor of Physiology* (1, 1947, 2, 1941)
- Seever, Maurice Harrison, Ph D , M D University of Michigan, Ann Arbor *Professor of Pharmacology* (1, 1933, 3, 1930)
- Segaloff, Albert, M D Alton Ochsner Medical Foundation, 3503 Prytania St , New Orleans, La *Director of Endocrine Research* (4, 1946)
- Seibert, Florence B , Ph D , Sc D , LL D Henry Phipps Institute, University of Pennsylvania, 7th and Lombard Sts , Philadelphia *Associate Professor of Biochemistry* (2, 1925)
- Seidell, Atherton, M S , Ph D 2301 Connecticut Ave , Washington, D C *Special Expert, National Institute of Health* (2, 1924)
- Seifter, Joseph, M D Wyeth Institute of Applied Biochemistry, Philadelphia, Pa *Director of Research* (3, 1940)
- Seifter, Sam, M S , Ph D 350 Henry St , Brooklyn 2, N Y *Assistant Professor of Biochemistry, Long Island College of Medicine* (2, 1946)
- Selkurt, Ewald E , Ph D School of Medicine, Western Reserve University, Cleveland 6, O *Senior Instructor in Physiology* (1, 1945)
- Selle, Wilber Arthur, Ph D Medical School, University of Texas, Galveston *Professor of Physiology* (1, 1938)
- Sellers, E A , M D , Ph D Department of Physiology, University of Toronto, Ontario, Canada *Assoc Professor of Physiology, Assoc Professor in the Banting and Best Dept of Medical Research* (1, 1947)
- Selye, Hans, M D , Ph D , D Sc , F R S (c ) Inst of Experimental Medicine and Surgerv, Univ of Montreal, Montreal, Canada *Professor and Director* (1, 1934)
- Sendroy, Julius, Jr , M A , Ph D Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md *Chief Chemist* (2, 1928)
- Sevag, M G , Ph D Department of Bacteriology, University of Pennsylvania School of Medicine, Philadelphia *Associate Professor of Biochemistry in Bacteriology* (6, 1941)
- Sevringhaus, Elmer L , M A , M D Hoffmann-LaRoche, Inc , Nutley 10, N J *Director of Clinical Research, Clinical Professor of Medicine, New York Medical College, Director, Endocrinology and Metabolism, Jersey City Med Center* (2, 1923, 5, 1939)
- Shaffer, Morris F , D Phil Department of Pathol-

- ogy and Bacteriology, School of Medicine, Tulane University of Louisiana, New Orleans *Head of Department* (4, 1939, 6, 1937)
- Shaffer, Philip A**, Ph D Washington University Medical School, St Louis 40, Mo *Distinguished Service Professor of Biological Chemistry, Member National Academy of Sciences* (2, 1906, 5, 1935)
- Shanes, Abraham M**, M S, Ph D Dept of Physiology and Biophysics, Georgetown Univ School of Medicine, Washington 7, D C (1, 1946)
- Shank, Robert E**, M D Washington University Sch of Med, St Louis, Mo *Prof of Preventive Med* (2, 1947)
- Shannon, James A**, Ph D, M D Squibb Institute for Medical Research, New Brunswick, N J *Director, Squibb Institute for Medical Research* (1, 1933, 3, 1945)
- Shapiro, Herbert**, Ph D Henry Phipps Institute, 7th & Lombard St, Philadelphia 47, Pa *Res Associate Bethesda 14, Md* (1, 1937)
- Sharpless, George R**, M S, Sc D Lederle Laboratories, Pearl River, N Y *Research Biochemist* (5, 1942)
- Shaw, J C**, M S, Ph D Department of Dairy Husbandry, University of Maryland, College Park, Md *Professor* (1, 1947)
- Shaw, James H**, Ph D Harvard School of Dental Medicine, Boston, Mass *Assoc in Nutrition* (5, 1948)
- Shaw, Myrtle**, M S, Ph D 11 S Lake Ave, Albany, N Y *Senior Bacteriologist, Division of Laboratories and Research, New York State Department of Health* (6, 1937)
- Shay, Harry**, M D Temple University School of Medicine, Philadelphia, Pa *Director, Fels Research Institute and Clinical Professor of Medicine* (1, 1944)
- Shear, M J**, Ph D National Cancer Institute, Bethesda, Md *Chief Biochemist and Chairman, Chemotherapy Section* (2, 1930)
- Sheard, Charles, A M**, Ph D Mayo Foundation, Rochester, Minn *Chief of the Division of Physics and Biophysical Research and Professor of Physiological Optics and Biophysics, University of Minnesota* (1, 1925)
- Sheehan, Donal**, M D, D Sc The Commonwealth Fund, New York City *General Director* (1, 1938)
- Shelesnyak, M C**, Ph D Office of Naval Research, Med Sciences Div, Washington, D C *Head, Human Ecology Branch* (1, 1948)
- Shelley, Walter Brown**, Ph D, M D University of Pennsylvania School of Medicine *Assistant Instructor of Dermatology* (1, 1946)
- Shemin, David, A M**, Ph D Columbia University, College of Physicians and Surgeons, 630 W 168th St, New York City *Assistant Professor of Biochemistry* (2, 1944)
- Sheppard, Fay**, M S University of Oklahoma Medical School, Oklahoma City *Instructor in Biochemistry* (2, 1936)
- Sherman, Henry C**, M A, Ph D, Sc D Columbia University, New York City *Mitchell Professor Emeritus of Chemistry, Member, National Academy of Sciences* (1R, 1923, 2, 1906, 5, 1933)
- Sherwin, Carl Paxson**, Sc D, M D, Dr P H, LL D 6 Carstensen Rd, Scarsdale, N Y *Director of Metabolic Service, St Vincent's Hospital, Associate Physician, French Hospital* (1, 1919, 2, 1917)
- Sherwood, Noble P**, Ph D, M D 517 Snow Hall, Univ of Kansas, Lawrence, Kan *Professor of Bacteriology* (6, 1928)
- Sherwood, Thomas Cecil**, M A, Ph D, M D 1824 Robert St, New Orleans, La *Southern Baptist Hospital Staff Member, Internal Medicine* (1, 1938)
- Shettles, Landrum B**, Ph D, M D College of Physicians and Surgeons, Columbia Presbyterian Medical Center, Box 330, 622 W 168th St, New York City (1, 1946)
- Shideman, Frederick E**, B A, Ph D Dept of Pharmacology, University of Michigan, Ann Arbor *Instructor of Pharmacology* (3, 1944)
- Shimkin, Michael Boris**, M D University of California Medical School, San Francisco *Director, Laboratory of Experimental Oncology* (4, 1940)
- Shipley, Reginald A**, M D Western Reserve University School of Medicine, Cleveland 6, O *Assistant Professor of Medicine* (1, 1945)
- ShIPLEY, Robert E**, M D Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind (1, 1945)
- Shive, William**, Ph D Univ of Texas, Austin, Texas *Assoc Professor of Chemistry* (2, 1948)
- Shlaer, Simon**, M A, Ph D Box 1663, Los Alamos, New Mexico (1, 1938)
- Shock, Nathan W**, Ph D Section on Gerontology, U S Public Health Service, Baltimore City Hospitals, Baltimore 24, Md *Chief, Section on Gerontology, U S Public Health Service, National Institute of Health, Bethesda, Md* (1, 1942)
- Shoemaker, Harold A**, M S, Ph D University of Oklahoma School of Medicine, 801 E 13th St, Oklahoma City *Assistant Dean, Professor of Pharmacology* (3, 1941)
- Shope, Richard E**, M D Ridge Road, Kingston, N J (4, 1934)
- Shorr, Ephraim**, B A, M D The New York Hospital, 525 East 68th St, New York City *Assistant Professor of Medicine, Cornell University Medical College, Assistant Attending Physician, The New York Hospital* (1, 1931, 3, 1942)
- Shrigley, E W**, PhD, M D Yale University School of Medicine, Dept of Bacteriology and

- Immunology, New Haven, Conn *Asst Professor of Bacteriology* (6, 1946)
- Shwartzman, Gregory, M D 230 E 50th St, New York City *Head of Department of Bacteriology, Mount Sinai Hospital, Professor of Bacteriology, Columbia University* (4, 1929, 6, 1930)
- Sichel, F J M, Sc M, Ph D College of Medicine, University of Vermont, Burlington *Associate Professor of Physiology* (1, 1939)
- Sickles, Grace M, B A 2201 Twelfth St, Troy, N Y *Associate Bacteriologist, Division of Laboratories and Research, New York State Department of Health* (6, 1932)
- Sickles, Gretchen R, A B Division of Laboratories and Research, New York State Department of Health, Albany, N Y *Assistant Bacteriologist* (6, 1937)
- Siebenmann, Charles O, Ch E, D Eng Connaught Medical Research Laboratories, Univ of Toronto, Toronto 5, Ontario, Canada *Research Associate* (3, 1946)
- Siebert, Walter J, M D Lutheran Hospital, St Louis, Mo *Director of Laboratories and Pathologist* (4, 1932)
- Silber, Robert H, Ph D Merck Institute for Therapeutic Research, Linden, N J *Department Head* (2, 1948)
- Silberberg, Martin, M D Snodgras Laboratory of Pathology, City Hospital, 1430 Carrol St, St Louis 4, Mo *Instructor in Pathology, Washington University, School of Medicine* (4, 1944)
- Silberberg, Ruth, M D Snodgras Laboratory of Pathology, City Hospital, 1430 Carroll St, St Louis 4, Mo *Instructor in Pathology, Washington University, School of Medicine* (4, 1944)
- Silvette, Herbert, M S, Ph D University of Virginia Medical School, University, Va (IR, 1943, 3, 1940)
- Simmonds, Sofia, Ph D Yale Univ, Osborn Botanical Laboratory, New Haven, Conn *Instructor in Microbiology* (2, 1948)
- Simon, Frank A, M D 332 West Broadway, Louisville, Ky (6 1934)
- Simonds, James P, Ph D, M D Northwestern University Medical School, 234 E Pearson St, Chicago 2, Ill *Emeritus Professor of Pathology* (4, prior to 1920)
- Simonsen, Ernst, M D c/o Laboratory of Physiological Hygiene, Stadium South Tower, University of Minnesota, Minneapolis 14 *Associate Professor of Physiology* (1, 1941)
- Simpson, Miriam E, M A, Ph D, M D Div of Anatomy, Univ of Calif, Berkeley, Calif *Professor of Anatomy* (1, 1946)
- Sinclair, Robert Gordon, Ph D, F R S C Queen's University, Kingston, Ont, Canada *Professor of Biochemistry* (2, 1931)
- Singal, Sam A, Ph D Univ of Georgia School of Medicine, Augusta, Ga *Asst Professor of Biochemistry* (2, 1948)
- Singer, Thomas P, Ph D Western Reserve Univ, Cleveland, Ohio *Asst Professor of Biochemistry* (2, 1948)
- Sizer, Irwin W, Ph D Massachusetts Institute of Technology, Cambridge *Associate Professor of Physiology* (1, 1944)
- Skinner, John Taylor, M S, Ph D c/o The Grapette Co, Camden, Arkansas *Chief Chemist* (2, 1946)
- Slaughter, Donald, M D Univ of South Dakota, School of Medical Sciences, Vermillion, S D *Dean* (3, 1938)
- Slonaker, James R, Ph D 334 Kingsley Ave, Palo Alto, Calif *Professor of Physiology, Leland Stanford Junior University* (1, 1917)
- Smadel, Joseph Edwin, M D Department of Virus and Rickettsial Diseases, Army Medical Department, Research and Graduate School, Washington 12, D C *Scientific Director* (4, 1940, 6, 1937)
- Small, James C, M D 101 S 39th St, Philadelphia, Pa *Associate in Medicine, Graduate School of Medicine, University of Pennsylvania* (4, 1927)
- Smetana, Hans F, M D Army Institute of Pathology, 7th St, and Independence Ave, Washington 25, D C (4, 1934)
- Smith, Arthur H, M S, Ph D Wayne University College of Medicine, Detroit 26, Mich *Professor of Physiological Chemistry* (1, 1923, 2, 1921, 5, 1933)
- Smith, Austin Edward, M D, C M, M Sc (Med) American Medical Association, 535 N Dearborn St, Chicago, Ill *Acting Secretary of the Council on Pharmacy and Chemistry, American Medical Association, Research Associate (Instructor), Dept of Pharmacology, University of Chicago* (3, 1942)
- Smith, Clarence A, M S, Ph D Standard Brands, Inc, 595 Madison Ave, New York City *Technical Director, Agricultural Department* (1, 1921)
- Smith, David T Duke Hospital, Durham, N C *Professor of Bacteriology and Associate Professor of Medicine* (5, 1943)
- Smith, Dietrich Conrad, A M, Ph D University of Maryland School of Medicine, Lombard and Greene Sts, Baltimore *Associate Professor of Physiology* (1, 1937)
- Smith, Douglas C, M S, Ph D Argonne National Laboratory, P O Box 5207, Chicago 80, Ill (1, 1947)
- Smith, Edwin L, Ph D Univ of Texas Dental Sch, Houston, Texas *Professor of Physiology* (1, 1948)
- Smith, Elinor Van Dorn, Ph D 5 Middle St,

- Hadley, Mass *Associate Professor of Bacteriology, Smith College* (6, 1940)
- Smith, Elizabeth R B, Ph D c/o Dr Paul K Smith, 1335 H St, N W, Washington 5, D C (2, 1938)
- Smith, Emil L, Ph D School of Medicine, Univ of Utah, Salt Lake City 1, Utah *Associate Professor of Biochemistry* (2, 1946)
- Smith, Erma A, M A, Ph D, M D College Station, Durham, N C (1, 1928)
- Smith, George H, M A, Ph D, M A (hon), Sc D School of Medicine, Yale University, New Haven, Conn *Professor of Immunology and Assistant Dean, Chairman, Department of Bacteriology, Yale University* (6, 1918)
- Smith, H P, M S, M D Columbia Univ, Coll of Physicians and Surgeons, 630 West 168th St, New York 32, N Y *Delafield Professor of Pathology* (1, 1937, 4, 1925)
- Smith, Homer W, M S (hon), Sc D 177 First Ave, New York City *Professor of Physiology, New York University College of Medicine, Member, National Academy of Sciences* (1, 1923, 2, 1930)
- Smith, Janice M, M S, Ph D Department of Home Economics, University of Illinois, Urbana, Ill *Professor and Chief of Nutrition* (5, 1947)
- Smith, John R, A M, M D Washington University School of Medicine, St Louis 10, Mo *Assistant Professor of Medicine* (1, 1947)
- Smith, Lawrence Weld, M D 119 E 26th St, New York 10, N Y (4, 1927)
- Smith, Lee Irvin, A M, Ph D School of Chemistry, University of Minnesota, Minneapolis *Professor and Chief, Division of Organic Chemistry* (2, 1942)
- Smith, Margaret Cammack, Ph D El Encanto Estates, Tucson, Arizona (2, 1935, 5, 1933)
- Smith, Maurice I, M D National Institute of Health, Bethesda 14, Md *Principal Pharmacologist, U S Public Health Service* (1, 1920, 3, 1916)
- Smith, Paul K, Ph D Department of Pharmacology, George Washington Univ School of Medicine, 1335 H St, N W, Washington 5, D C *Professor of Pharmacology and Executive Officer of the Department* (2, 1937, 3, 1937)
- Smith, Paul W, M S, Ph D School of Medicine, University of Oklahoma, 801 E 13th St, Oklahoma City *Associate Professor of Pharmacology* (1, 1933)
- Smith, Philip Edward, M S, Ph D 630 W 168th St, New York City *Professor of Anatomy, Columbia University, Member of the National Academy of Sciences* (1, 1923)
- Smith, Ralph G, M D, Ph D Tulane University, Station 20, New Orleans, La *Professor of Pharmacology* (3, 1929)
- Smith, R Blackwell, Jr, B S, M S, Ph D Medical College of Virginia, Richmond 19, Va *Lecturer in Pharmacology* (3, 1914)
- Smith, Sedgwick E, Ph D Dept Animal Husbandry, Cornell University, Ithaca, N Y *Animal Physiologist* (5, 1915)
- Smith, Susan Gower, M A Duke University, Durham, N C *Associate, Department of Medicine and Nutrition, School of Medicine* (5, 1939)
- Smith, Wilbur Kenneth, M D University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y *Associate Professor of Anatomy* (1, 1939)
- Smith, Willie W, M A, Ph D Bethesda, Md *Associate Physiologist, National Institute of Health* (1, 1941)
- Smithburn, Kenneth C, M D The Rockefeller Foundation, 49 W 49th St, New York City *Staff Member, International Health Division* (6, 1937)
- Smolens, Joseph, B S Wyeth Research Inst, 900 N Broad St, Philadelphia, Pa *Head, Dept of Immunology* (6, 1943)
- Smythe, C V, M S, Ph D 5000 Richmond St, Philadelphia, Pa *Head of Biochemistry, Rohm & Haas Company* (2, 1934)
- Snape, William J, M D Jefferson Med College of Philadelphia, Philadelphia, Pa *Assoc in Physiology* (1, 1948)
- Snell, Albert M, M D Mayo Clinic, Rochester, Minn *Head of Section on Medicine at Mayo Clinic, Professor in Medicine, Mayo Foundation Graduate School, University of Minnesota* (4, 1930)
- Snell, Esmond E, M A, Ph D University of Wisconsin, Madison 6 *Professor of Biochemistry* (2, 1942, 5, 1916)
- Snyder, Franklin Faust, M D Boston Lying In Hospital, Boston, Mass (1, 1936)
- Sobel, Albert E, M A, Ph D Jewish Hospital of Brooklyn, Brooklyn, N Y *Head, Dept of Chemistry, Adjunct Professor of Chemistry, Polytechnic Institute of Brooklyn* (2, 1939)
- Sobotka, Harry H, Ph D Mount Sinai Hospital, Fifth Ave and 100th St, New York City *Head, Department of Chemistry* (2, 1932, 5, 1933)
- Solandt, Donald Young, M A, M D, Ph D University of Toronto, Toronto, Ont, Canada *Professor of Physiology in charge of Biophysics, Faculty of Medicine, Professor of Physiological Hygiene and Head of Department, School of Hygiene* (1, 1937)
- Soley, Mayo H, M D University of California Medical School, The Medical Center, San Francisco *Associate Professor of Medicine and Assistant Dean* (1, 1943)
- Sollmann, Torald, M D Sc D, (hon), LL D 14327 Superior Road, Cleveland, Ohio *Pro-*

- fessor of Pharmacology, Emeritus, Western Reserve Univ (1R, 1902, 2, 1906, 3, 1908)
- Solotorovsky, Morris, Ph D 203 W 5th St, Plainfield, N J Merck Institute for Therapeutic Research, Rahway, N J, Research Associate in Chemotherapy (6, 1946)
- Somogyi, Michael, Ph D 216 S Kingshighway, St Louis, Mo Biochemist, Jewish Hospital of St Louis (2, 1927)
- Soskin, Samuel, M D, M A, Ph D Michael Reese Hospital, 29th St and Ellis Ave, Chicago 16, Ill Medical Director and Director of Research Institute, Michael Reese Hospital, Dean, Michael Reese Hospital Postgraduate School, Professorial Lecturer in Physiology, University of Chicago (1, 1930, 5, 1933)
- Soule, Malcolm H, Sc D, LL D, Ph D University of Michigan, Ann Arbor Professor of Bacteriology, and Chairman of the Department of Bacteriology (4, 1927, 6, 1925)
- Spain, Will C, M D, F A C P 116 E 53rd St, New York City Clinical Professor of Medicine, Post Graduate Medical School, Columbia University (6, 1923)
- Spealman, C R, M A, Ph D Dept of Physiology Univ of Pennsylvania School of Medicine, Philadelphia, Pa (1, 1940)
- Specht, Heinz, Ph D National Institute of Health, Rockville Pike, Bethesda, Md Associate Research Physiologist (1, 1941)
- Sperry, Roger W, Ph D Dept of Anatomy, University of Chicago, Chicago 37, Ill (1, 1945)
- Sperry, Warren M, M S, Ph D 722 W 168th St, New York City Principal Research Biochemist, New York State Psychiatric Institute (2, 1929, 5, 1933)
- Spiegel, Ernest A, M D Temple University School of Medicine, Broad and Ontario Sts, Philadelphia, Pa Professor of Experimental Neurology (1, 1936)
- Spiegel-Adolf, Mona, M D Temple University School of Medicine, Broad at Ontario Sts, Philadelphia, Pa Professor and Head of Department of Colloid Chemistry (2, 1933)
- Spiegelman, Sol, Ph D University of Minnesota, Medical School, Dept of Physiology, Minneapolis 14, Minn (1, 1946)
- Spies, Tom D, M D Hillman Hospital, Birmingham, Ala Director, Nutrition Clinic (3, 1941, 4, 1940, 5, 1938)
- Spink, Wesley W, M D University of Minnesota Hospital, Minneapolis Associate Professor of Medicine, University of Minnesota Medical School (3, 1940, 4, 1940, 6, 1940)
- Spohn, Adelaide, M S, Ph D Elizabeth McCormick Memorial Fund, 848 N Dearborn St, Chicago, Ill Nutritionist (5, 1933)
- Spoor, Herbert J, Ph D Bristol Myers Co, New York City Asst Director, Med Div (1, 1945)
- Sproul, Edith E, M D Dept of Pathology, American Univ of Beirut, Beirut, Lebanon Professor of Pathology (4, 1941)
- Sprunt, Douglas H, M D, M S Univ of Tennessee, Memphis Professor of Pathology, Head of Dept of Pathology and Bacteriology (4, 1934, 6, 1936)
- Stadie, William C, M D 821 Maloney Clinic, 36th and Spruce Sts, Philadelphia, Pa Professor of Research Medicine, University of Pennsylvania (2, 1922)
- Stainsby, Wendell J M D Geisinger Memorial Hospital, Danville, Pa Chief Physician (6, 1930)
- Stanley, Wendell M, M S, Ph D, Sc D, LL D University of California, Berkeley, Calif Prof of Biochemistry and Director of Virus Lab (2, 1936)
- Stannard, James Newell, Ph D National Institute of Health, U S Public Health Service, Bethesda 14 Md Senior Pharmacologist (1, 1938)
- Stare, Fredrick J, Ph D, M D 695 Huntington Ave, Boston 15, Mass Professor of Nutrition, Harvard University (2, 1937, 5, 1942)
- Starr, Isaac, B S, M D Hospital of the University of Pennsylvania, Philadelphia Hartzell Prof of Res Therapeutics (1, 1929, 3, 1942)
- Stavraky, George W, M D, C M, M Sc Medical School, University of Western Ontario, London, Ont, Canada Associate Professor of Physiology (1, 1937, 3, 1944)
- Stead, Eugene A, Jr, M D Department of Medicine, Duke University, Durham, N C (1, 1945)
- Stearns, Genevieve, Ph D College of Medicine, State University of Iowa, Iowa City Research Professor of Pediatrics (2, 1932, 5, 1937)
- Steel, Matthew, Ph D Long Island College of Medicine, 350 Henry St, Brooklyn, N Y Professor of Biological Chemistry (2, 1909)
- Steele, J Murray, M D Third (N Y U) Research Division Goldwater Memorial Hosp, Welfare Island, New York City Associate Professor of Medicine, New York University, Director 3rd (New York University) Medical Division of Welfare Hospital (1, 1936)
- Steenbock, Harry, M S, Ph D, Sc D University of Wisconsin, Madison Professor of Biochemistry (2, 1912, 5, 1933)
- Steggerda, F R, M A, Ph D 416 Natural History Building, University of Illinois, Urbana Associate Professor of Physiology (1, 1934)
- Stehle, Raymond Louis, A M, Ph D Faculty of Medicine, McGill University, Montreal, Canada Professor of Pharmacology (2, 1920, 3, 1922)
- Steigmann, Frederick, M S, M D 348 S Hamlin

- Ave, Chicago, Ill *Associate in Medicine, College of Medicine, University of Illinois, Associate Attending Physician, Cook County Hospital* (3, 1942)
- Steiman, S E, M A, Ph D, M D 23 Broad St, Lynn, Mass *Assistant Physician, Metropolitan State Hospital, Waltham, Mass* (1, 1939)
- Stein, George J, M S, Ph D *Army Medical Center, Washington, D C Chief, Research Section, Veterans Division, Army Medical Department of Research and Graduate School* (6, 1947)
- Stein, William Howard, Ph D *The Rockefeller Institute for Medical Research, 66th and York Ave, New York 21, N Y Associate in Chemistry* (2, 1946)
- Steinbach, H Burr, M A, Ph D *Dept of Zoology, University of Minnesota, Minneapolis* (1, 1934)
- Steinberg, Bernhard, M D *Toledo Hospital Institute of Medical Research, Toledo, O Director of the Toledo Hospital Institute of Medical Research, Director of Clinical and Morbid Pathological Laboratories, The Toledo Hospital, Surgeon, U S P H (inactive)* (4, 1928, 6, 1946)
- Steiner, Paul E, M D *The University of Chicago, Chicago, Ill Professor of Pathology* (4, 1939)
- Steinhardt, Jacinto, A M, Ph D 1548 East-West Highway, Silver Spring, Md *Director, Operations Evaluation Group, Mass Institute of Technology* (2, 1939)
- Steinhaus, Arthur H, M S, Ph D, M P E 5315 Drexel Ave, Chicago, Ill *Professor of Physiology, George Williams College, Hyde Park* (1, 1928)
- Stekol, Jakob A, M A, D Sc *The Lankenau Hospital Research Institute, Philadelphia 30, Pa Associate Member* (2, 1936)
- Stern, Kurt G, Ph D *Polytechnic Institute, Brooklyn, N Y Adjunct Professor of Biochemistry* (2, 1938)
- Stetten, DeWitt, Jr, M D, Ph D *Public Health Res Inst of the City of New York, New York City Chief, Division of Nutrition and Physiology* (2, 1944)
- Stetten, Marjorie Roloff, Ph D *Public Health Res Inst of the City of New York New York City Associate, Div of Nutrition and Physiology* (2, 1947)
- Stevens, S Smith, Ph D *Emerson Hall, Harvard University, Cambridge, Mass Assistant Professor of Psychology* (1, 1937)
- Stewart, Dorothy R, M S, Ph D *Rockford College, Rockford, Ill* (1, 1947)
- Stewart, Fred W, M D *Memorial Hospital, 444 E 68th St, New York City Pathologist, Associate Professor of Surgical Pathology, Cornell Medical School, Pathologist, New York State Department of Public Health, Division of Laboratories and Research* (4, 1928)
- Stewart, Harold L, M D *The National Cancer Institute, Bethesda, Md Chief, Pathology Section* (4, 1936)
- Stewart, Winifred Bayard, M D, M A 1930 Spruce St, Philadelphia, Pa *Professor of Neurology, Woman's Medical College of Pennsylvania* (1, 1941)
- Stickney, J Clifford, M S, Ph D *West Virginia University School of Medicine, Morgantown Assistant Professor of Physiology* (1, 1944)
- Stiebeling, Hazel K, M A, Ph D *United States Department of Agriculture, Washington, D C Chief, Bureau of Human Nutrition and Home Economics* (5, 1933)
- Stier, Theodore J. B, Ph D *Indiana University Medical School, Bloomington Associate Professor of Physiology* (1, 1938)
- Still, Eugene U, Ph D % Strong Cobb & Co, 2654 Lisbon Rd, Cleveland, O (1, 1929)
- Stillman, Ernest G, M D 45 E 75th St, New York City (6, 1930)
- Stummel, Benjamin F, Ph D *Rees Stealy Medical Research Fund, Ltd, 2001 Fourth Avenue, San Diego, Calif Research Biochemist* (2, 1947)
- Stock, Aaron H, M D *School of Medicine, University of Pittsburgh, Pittsburgh, Penn Assistant Professor of Bacteriology and Immunology* (6, 1917)
- Stockton, Andrew Benton, M D 655 Sutter Street, San Francisco, Calif (3, 1931)
- Stoerk, Herbert C, M D *Merck Institute for Therapeutic Research, Rahway, N J Head, Department of Cancer Research* (4, 1948)
- Stohman, Edward F, LL B *National Institute of Health, Bethesda, Md Assoc Pharmacologist* (3, 1948)
- Stokinger, Herbert B, Ph D 250 Meigs Street, Rochester, N Y *Assoc Professor of Pharmacology and Toxicology* (6, 1947)
- Stokstad, E L Robert, Ph D *Lederle Laboratories, Pearl River, N Y Chemist* (2, 1947, 5, 1942)
- Stoland, O O, M S, Ph D 1845 Learned Ave, Lawrence, Kan *Professor of Physiology and Pharmacology, University of Kansas* (1, 1913)
- Stone, William E, Ph D *Department of Physiology, University of Wisconsin, Madison Assistant Professor of Physiology* (1, 1945)
- Stormont, Robert T, Ph D, M D *Food and Drug Administration, Washington, D C Medical Director, Professional Lecturer in Pharmacology, Georgetown Univ Medical School* (3, 1941)
- Storvick, Clara A, M S, Ph D *School of Home Economics, Oregon State College, Corvallis,*

- Ore Associate Professor of Foods and Nutrition (5, 1947)
- Stolz, Elmer H , Ph D University of Rochester School of Medicine and Dentistry, Rochester 7, N Y Professor of Chemistry (2, 1939)
- Stoughton, Roger W , M S , Ph D Mallinckrodt Chemical Works, 3600 N Second St , St Louis, Mo Research Chemist (3, 1939)
- Strong, Frank M , M A , Ph D Department of Biochemistry, University of Wisconsin, Madison Professor of Biochemistry (2, 1941)
- Struck, Harold Carl, Ph D School of Medicine, The Creighton University, 302 N 14th St , Omaha 2, Nebr (1, 1940)
- Stuart, Charles A , M Sc , Ph D 372 Lloyd Ave , Providence, R I Associate Professor of Biology, Brown University (6, 1935)
- Sturgis, Cyrus Cressey, M D Simpson Memorial Institute, Ann Arbor, Mich Director, Thomas Henry Simpson Memorial Institute for Medical Research, Chairman, Department of Medicine, University Hospital, and Professor of Medicine, University of Michigan (4, 1927)
- Sturkie, Paul D , Ph D Rutgers Univ , New Brunswick, N J Assoc Res Specialist in Poultry Husbandry and Assoc Prof of Poultry Husbandry (1, 1948)
- Stutzman, Jacob W , Ph D , M D Boston University School of Medicine, Boston, Mass Assoc Professor of Pharmacology (1, 1946, 3, 1948)
- Sugg, John Y , Ph D Cornell University Medical College, 1300 York Ave , New York City Associate Professor of Bacteriology and Immunology (6, 1938)
- Sulkin, S Edward, Ph D Southwestern Medical Foundation, Dallas, Texas Professor and Chairman, Dept of Bacteriology and Immunology (6, 1944)
- Sullivan, Michael Xavier, Ph D Chemo-Medical Research Institute, Georgetown University, 37th & O Sts , N W , Washington, D C Director and Research Professor of Chemistry (2, 1909)
- Sulzberger, Marion B , M D 999 5th Ave , New York City Associate Clinical Professor of Dermatology and Syphilology, N Y Post-Graduate Medical School of Columbia Univ , Assoc Director, Skin and Cancer Unit of N Y Post Graduate Hospital (6, 1936)
- Summerson, William H , Ph D Medical Division, Army Chemical Center, Md Chief, Biochemistry Section (2, 1942)
- Sumner, J B , A M , Ph D Cornell University, Ithaca, N Y Director, Enzyme Chemistry Lab (2, 1919)
- Sunderman, F William, M D , Ph D Cleveland Clinic Found , Cleveland, Ohio Head, Dept of Clin Pathology, Professor of Clin Pathology, Bunts Institute, Cleveland Clinic (2, 1931)
- Sundstroem, Edward S , M D University of California, Berkeley Professor of Biochemistry, Emeritus (2, 1919)
- Sure, Barnett, M S , Ph D University of Arkansas, Fayetteville Head of Department and Professor of Agricultural Chemistry (2, 1923, 5, 1933)
- Sutherland, George F , CM , M D , M Sc Duke University School of Medicine, Durham, N C (1, 1939)
- Sutton, T Scott, M Sc , Ph D Ohio State University, Columbus Professor, Ohio State University, Associate, Ohio Agricultural Experiment Station, Director, Institute of Nutrition and Food Technology (5, 1936)
- Svirbel, Joseph L , Ph D Monsanto Chemical Co , Central Research Department, 1601 W First St , Dayton 7, Ohio Pharmacologist (3, 1945)
- Swain, Robert E , M S , Ph D , LL D 634 Mirada Ave , Stanford University, Calif Professor Emeritus of Chemistry (2, 1909)
- Swann, Howard G , M S , Ph D Dept of Physiology, University of Texas Medical School, Galveston Assistant Professor of Physiology Captain, Aero Medical Laboratory, Wright Field, Dayton, O (1, 1940)
- Swanson, Pearl P , M S , Ph D Iowa State College, Ames Professor of Foods and Nutrition, Dept of Foods and Nutrition (5, 1933)
- Swanson, William W , M S , M D 2376 E 71st St , Chicago, Ill Assistant Professor of Pediatrics, Northwestern University (2, 1938)
- Sweeney, H Morrow, M S , Ph D Aero Medical Laboratory, Wright Field, Dayton, Ohio (1, 1939)
- Swift, Homer, M D , D Sc 66th St and York Ave , New York City Member, Rockefeller Institute for Medical Research, Physician to The Hospital of The Rockefeller Institute for Medical Research (6, 1920)
- Swift, Raymond W , M S , Ph D Pennsylvania State College, State College Professor, Department of Animal Nutrition (5, 1934)
- Swingle, Wilbur Willis, Ph D Princeton University, Princeton, N J Professor of Biology (1, 1924)
- Swinyard, Ewart A , Ph D Univ of Utah, Salt Lake City, Utah Professor of Pharmacy, Sch of Pharmacy, Lecturer in Pharmacology, Sch of Medicine (3, 1948)
- Sydenstricker, V P University of Georgia School of Medicine, Augusta Professor of Medicine (5, 1944)
- Sykes, Joseph F , M S A , Ph D U S Dept of Agriculture, Bureau of Dairy Industry, Beltsville, Md Physiologist (1, 1942)
- Syvertson, Jerome T , M D University of Minnesota, Minneapolis, Minn Professor and Head,

- Dept of Bacteriology and Immunology* (4, 1940, 6, 1947)
- Szego, Clara M**, M S, Ph D University of California, Medical School, Los Angeles *Assistant Clinical Professor of Biophysics* (1, 1946)
- Szepsenwol, Josel**, M D Emory Univ Sch of Med, Emory University, Ga *Asst Professor of Anatomy* (1, 1948)
- Tabor, Herbert**, M D Division of Physiology, National Institutes of Health, Bethesda 14, Md *Senior Assistant Surgeon, USPHS* (3, 1947)
- Tager, Morris** Western Reserve Univ, Cleveland, Ohio *Dept of Microbiology* (6, 1948)
- Tainter, M L**, M A, M D Sterling-Winthrop Research Institute, 33 Riverside Ave, Rennselaer, N Y *Director* (1, 1929, 3, 1927)
- Talbot, Samuel Armstrong**, A M, M S, Ph D Wilmer Institute, Johns Hopkins Hospital, Baltimore, Md *Instructor in Physiological Optics, Johns Hopkins University* (1, 1940)
- Taliaferro, William H**, Ph D. Department of Bacteriology, University of Chicago, Chicago, Ill *Eliakim H Moore Distinguished Service Professor of Parasitology and Dean of the Division of Biological Sciences* (6, 1930)
- Tannenbaum, Albert**, M D Michael Reese Hospital, 29th St & Ellis Ave, Chicago, Ill *Director, Department of Cancer Research* (4, 1942)
- Tarver, Harold**, M S, Ph D Division of Biochemistry, University of California, Berkeley, Calif *Assistant Professor of Biochemistry* (2, 1947)
- Tashiro, Shiro**, Ph D, M D College of Medicine, University of Cincinnati, Cincinnati, O *Professor of Biochemistry* (1, 1913, 2, 1913)
- Tatum, Arthur L**, M S, Ph D, M D Service Memorial Institute, University of Wisconsin, Madison *Professor of Pharmacology* (1, 1913, 3, 1919)
- Tatum, Edward L**, M S, Ph D School of Biological Sciences, Stanford University, Stanford University, Calif *Professor of Biology* (2, 1947)
- Tauber, Henry**, Ph D V D Research Laboratory, U S Marine Hospital, Staten Island, N Y *Biochemist, U S Public Health Service* (2, 1933)
- Taylor, A N**, Ph D Univ of Oklahoma Sch of Med, Oklahoma City, Okla *Asst Professor and Chairman, Dept of Physiology* (1, 1948)
- Taylor, Alonzo E**, M D General Mills, Inc 400 2nd Ave S, Minneapolis, Minn *Director of Research Director Emeritus, Food Research Institute, Stanford University* (5, 1933)
- Taylor, Alton R**, Ph D Parke, Davis Co, Detroit 32, Mich *Senior Research, Research Division* (2, 1947, 6, 1943)
- Taylor, Craig L**, M A, Ph D Dept of Engineering, Univ of Calif, Los Angeles, Calif *Associate Professor of Engineering* (1, 1945)
- Taylor, Fred A**, Ph D 320 E North Ave, N S, Pittsburgh, Pa *Biochemist, Singer Memorial Laboratory* (2, 1933)
- Taylor, Haywood M**, M S, Ph D Duke University School of Medicine, Durham, N C *Associate Professor of Biochemistry and Toxicology, Biochemist and Toxicologist to Duke Hospital* (4, 1942)
- Taylor, Henry Longstreet**, Ph D University of Minnesota, School of Public Health, Minneapolis *Assistant Professor of Physiological Hygiene* (1, 1944)
- Taylor, John Fuller**, Ph D Washington University School of Medicine, Euclid and Kingshighway, St Louis, Mo *Assistant Professor of Biological Chemistry* (2, 1944)
- Taylor, M Wight** New Jersey Agricultural Experiment Station, New Brunswick *Assoc Biochem in Nutr, and Assoc Prof of Agr Biochem, Rutgers Univ* (5, 1944)
- Taylor, Norman Burke**, M D, F R S (Can), M R C S (Eng), L R C P (Lon), F R C S (Edin), F R C P (Can) University of Toronto, 5, Ontario, Ont, Canada *Professor of Physiology* (1, 1922)
- Taylor, Robert D**, M D Clinical Research Division, Cleveland Foundation, Cleveland 6, O *Member* (1, 1945)
- Teague, Robert S**, Ph D, M D Dept of Pharmacology and Physiology, Medical College of Alabama, Birmingham 5, Ala *Associate Professor of Physiology and Pharmacology* (3, 1942)
- Templeton, Roy D**, B S 5630 South Flores, San Antonio, Texas (1, 1935)
- Ten Broeck, Carl**, M D The Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, N J *Director of Dept of Animal and Plant Pathology* (4, 1932, 6, 1924)
- Tepperman, Jay**, M D Dept of Pharmacology, Syracuse University School of Medicine, Syracuse, N Y *Associate Professor of Pharmacology* (1, 1944)
- Terplan, Kornel L**, M D University of Buffalo, School of Medicine, Buffalo, N Y *Professor of Pathology* (4, 1935)
- Thannhauser, S J**, M D, Ph D Pratt Diagnostic Hospital, 30 Bennet St, Boston, Mass *Professor of Clinical Medicine, Tufts Medical School, Associate Chief, Pratt Diagnostic Hospital* (2, 1937)
- Thayer, Sidney Allen**, Ph D 1402 S Grand Blvd, St Louis 4, Mo *Associate Professor of Biochemistry, St Louis University School of Medicine* (2, 1933)
- Thienes, Clinton H**, A M, M D, Ph D University of Southern California School of Medi-



- cine, Los Angeles *Professor of Pharmacology* (3, 1928)
- Thomas, Arthur W, Ph D Columbia University, New York City *Professor of Chemistry* (2, 1924)
- Thomas, Byron H, M S, Ph D Iowa State College, Ames *Professor and Head, Animal Chemistry and Nutrition, Iowa Agricultural Experiment Station* (5, 1933)
- Thomas, Caroline Bedell, M D The Johns Hopkins Hospital, Baltimore, Md *Associate Professor of Medicine, Johns Hopkins University School of Medicine* (1, 1939)
- Thomas, J Earl, M S, M D Jefferson Medical College, Philadelphia, Pa *Professor of Physiology* (1, 1922, 3, 1924)
- Thompson, Marvin R, Ph C, B Sc, M Ph, (Hon) Ph D 67 Greenwich Ave, Stamford, Conn (3, 1944)
- Thompson, Randall L, Sc D, M D Indiana University, Medical Center, Indianapolis 7, Ind *Professor of Bacteriology* (6, 1937)
- Thompson, William R, Ph D 1 Darrock Rd, Delmar, N Y *Senior Biochemist, Division of Laboratories and Research, New York State Department of Health* (2 1934)
- Thomson, David Landsborough, M A, Ph D, F R S C McGill University, Montreal, Canada *Professor of Biochemistry and Dean of the Faculty of Graduate Studies and Research* (2, 1929)
- Thorn, George Widmer, M D Peter Bent Brigham Hospital, Boston, Mass *Professor of Medicine of Harvard University* (1, 1939)
- Thorpe, W T S D V M National Institutes of Health, Bethesda, Md *Veterinary Pathologist* (4, 1948)
- Tidwell, Herbert C, Ph D Southwestern Medical College, Dallas, Texas *Professor of Biochemistry and Chairman of the Dept* (2, 1948)
- Tillett, William S, M D, Sc D (hon) Department of Bacteriology, New York University College of Medicine, 477 First Ave, New York City *Professor of Medicine* (6, 1927)
- Tilt, Jennie, M S, Ph D Florida State University, Tallahassee *Professor of Physiological Chemistry and Nutrition* (5, 1937)
- Tipson, R Stuart, Ph D, D Sc Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa *Senior Fellow, Department of Research in Pure Chemistry* (2, 1937)
- Tipton, Samuel R, Ph D Department of Zoology, University of Tennessee, Knoxville *Professor of Zoology* (1, 1940)
- Tisdall, Frederick F, M D, F R C P (London), F R C P (C) Hospital for Sick Children, 55 College St, Toronto, Canada *Associate Professor of Pediatrics, Dept of Medicine, University of Toronto* (2, 1922, 5, 1933)
- Tislow, Richard, M D Schering Corporation, Bloomfield, N J *Director of Biological Laboratories* (1 1944)
- Titus, Harry W, A M, Ph D Lime Crest Research Lab, RFD #1, Newton, N J *Technical Counsellor and Director of Nutritional Research* (2, 1929, 5, 1933)
- Tobias, Julian M, M D University of Chicago, Chicago, Ill *Instructor in Physiology* (1, 1944)
- Tocantins, Leandro Maués, M D Jefferson Medical College, Philadelphia, Pa *Associate Professor of Medicine* (1, 1939)
- Todd, Wilbert R, Ph D Univ of Oregon Medical School, Portland, Ore *Assoc Professor of Biochemistry* (2, 5, 1948)
- Todhunter, Elizabeth Neige, M Sc, Ph D, University of Alabama, University *Professor of Nutrition* (5, 1939)
- Toennies, Gerrit, Ph D Lankenau Hospital, Philadelphia 30, Pa *Senior Member, Institute for Cancer Research* (2, 1934)
- Tolle, Chester D, Ph D Food and Drug Administration, Federal Security Agency, Washington, D C *Senior Biochemist* (5, 1942)
- Toman, James E P, Ph D Dept of Pharmacology and Physiology, Univ of Utah School of Medicine, Salt Lake City (1, 1945)
- Tomlinson, Wray Joseph, M D Fort Logan Veteran's Hospital, Denver, Colorado *Chief of Labs, Assist Prof of Pathology, Univ of Colorado School of Medicine* (4, 1945)
- Tompkins, Edna H, M D Laboratory of Applied Physiology, Yale University, 52 Hillhouse Ave, New Haven, Conn *Research Associate, Associate Professor* (4, 1941)
- Torda, Clara, Ph D, M D Cornell Univ Medical College, New York City *Research Fellow in Pharmacology* (1, 1943, 3, 1944)
- Toth, Louis A, M S, Ph D Dept of Physiology, Louisiana State University School of Medicine, New Orleans 13, La *Associate Professor of Physiology* (1, 1940)
- Totter, John R, M A, Ph D Univ of Arkansas School of Medicine, Little Rock, Ark *Associate Professor, Dept of Physiological Chemistry* (2, 1946)
- Tourtellotte, Dee, M S, D Sc Charles B Knox Gelatin Co, 4th and Erie Sts, Camden, N J *Head, Nutrition Laboratory* (5, 1935)
- Tower, Sarah Sheldon, M D, Ph D Johns Hopkins University, Baltimore, Md *Instructor in Psychiatry* (1, 1932)
- Trager, William, M D The Rockefeller Institute for Medical Research Department of Animal and Plant Pathology, Princeton, N J *Associate* (4, 1947)
- Traub, Frederick B, M D 205 East 82nd St, New York 28, N Y *Associate Bacteriologist, Jewish Hospital of Brooklyn* (6, 1946)

- Travell, Janet, M D** Cornell University Medical College, New York City *Assistant Professor of Clinical Pharmacology* (3, 1933)
- Travis, Lee Edward, A M, Ph D** University of Southern California, Los Angeles *Professor of Psychology and Director of the Psychological Center, Major, YAAF (Yuma, Ariz)* (1, 1929)
- Treadwell, Carleton R, M S, Ph D** Dept of Biochemistry, George Washington University School of Medicine, 1335 H St, N W, Washington, D C *Associate Professor of Biochemistry* (2, 1944)
- Treffers, Henry P, Ph D** Yale Medical School, Department of Immunology, New Haven, Conn *Associate Professor of Immuno-chemistry* (6, 1942)
- Trimble, Harry C, M S, Ph D, M D** 25 Shattuck St, Boston, Mass *Assistant Professor of Biological Chemistry, Harvard Medical School* (2, 1929, 5, 1936)
- Tuft, Louis H, M D** 1530 Locust St, Philadelphia, Pa *Assistant Professor of Medicine, Temple University Medical School, Chief of Clinic of Allergy and Applied Immunology, Temple University Hospital* (6, 1928)
- tum Suden, Caroline, M A, Ph D** Department of Physiology, Mount Holyoke College, South Hadley, Mass (1, 1936)
- Tunturi, Archie Robert, M S, Ph D** Univ of Oregon Medical School, Portland, Ore *Assistant Professor of Anatomy* (1, 1946)
- Tuohy, Edward B, M S, M D** Georgetown University Hospital, Washington, D C *Professor of Anesthesiology* (3, 1941)
- Turner, Abby H, Ph D** Mount Holyoke College, South Hadley, Mass *Professor of Physiology* (1, 1928)
- Tuttle, Waid Wright, M A, Ph D** State University of Iowa, Iowa City *Professor of Physiology* (1, 1925)
- Tweedy, Wilbur R, Ph D** Veterans Administration Hospital, Radioisotope Unit, Hines, Ill *Director* (2, 1931)
- Tyler, Albert, Ph D** California Institute of Technology, Pasadena, Calif *Assoc Professor of Embryology* (6, 1946)
- Tyler, David B, Ph D** Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Sts, Baltimore 5, Md *Member of Staff* (1, 1943)
- Umbreit, Wayne W, M Sc, Ph D** Merck Institute for Therapeutic Research, Rahway, N J *Head, Dept of Enzyme Chemistry* (2, 1947)
- Unna, Klaus R W, M D** 1853 W Polk St, Chicago 12, Ill *Assistant Professor, Dept of Pharmacology, Univ of Illinois Coll of Medicine* (1, 1941, 3, 1944, 5, 1942)
- Upton, Morgan, M A, Ph D** Dept of Psychology, Rutgers University, New Brunswick, N J (1, 1934)
- Urban, Frank, Ph D, M D** 302 Northern Building, Green Bay, Wis (2, 1932)
- Utter, Merton F, Ph D** Dept of Biochemistry, Western Reserve Univ, Cleveland, Ohio, *Associate Professor of Physiological Chemistry* (2, 1946)
- Vahlteich, Ella McCollum, M A, Ph D** 310 Walnut St, Engelwood, N J (5, 1933)
- Valle, J R, M D** Escola Paulista de Medicina, Caixa Postal 144-A, Sao Paulo, Brazil *Professor of Pharmacology, Escola Paulista de Medicina, Sao Paulo, Brazil* (3, 1947)
- Vanderscheer, James, Ch E** 136 Linwood Ave, Ridgewood, N J *Research Chemist, Lederle Labs* (6, 1946)
- Van Dyke, H B, Ph D, M D** 630 W 168th St, New York, N Y *Hosack Professor of Pharmacology, Columbia University, College of Physicians and Surgeons* (1, 1925, 3, 1927)
- van Harreveld, Anthonie, M A, M D** California Institute of Technology, Pasadena *Associate Professor of Physiology* (1, 1941)
- Van Liere, Edward J, M S, M D, Ph D** The School of Medicine, West Virginia University, Morgantown *Professor of Physiology and Dean* (1, 1927)
- Van Middlesworth, Lester, Ph D** Univ of Tennessee, Memphis, Tenn *Instr in Physiology* (1, 1948)
- Van Slyke, Donald D, Ph D, Sc D, M D** Rockefeller Institute for Medical Research, 66th St and York Ave, New York City *Emeritus Member, Member, National Academy of Sciences* (2, 1908)
- van Wagenen, Gertrude, Ph D** Yale University School of Medicine, New Haven, Conn *Associate Professor* (1, 1932)
- van Wagtenonk, Willem J, Ph D** Dept of Zoology, Indiana University, Bloomington, Ind *Associate Professor of Zoology* (2, 1946)
- Van Winkle, Walton, Jr, M D** American Medical Assn, 535 N Dearborn St, Chicago 10, Ill (3, 1939)
- Varney, Philip L** Washington Univ Sch of Med, St Louis, Mo *Asst Professor of Bacteriology* (6, 1948)
- Vars, Harry M, Ph D** Harrison Department of Surgical Research, University of Pennsylvania Medical School, Philadelphia *Assistant Professor of Physiological Chemistry* (2, 1935, 5, 1935)
- Velick, Sidney Frederick** Dept of Biochemistry, Washington Univ School of Medicine, Euclid Ave and Kingshighway, St Louis 10, Mo *Assistant Professor of Biochemistry* (2, 1946)
- Vennesland, Birgit, Ph D** Dept of Biochemistry,

- University of Chicago, Chicago, Ill Assistant Professor (2, 1944)
- Yenning, Eleanor H, M S, Ph D University Clinic, Royal Victoria Hospital, Pine Ave, Montreal, Quebec, Canada Assistant Professor of Medicine, McGill Univ (2, 1938)
- Vestling, Carl Swenson, Ph D Noyes Lab, Univ of Illinois, Urbana, Ill Assistant Professor of Biochemistry (2, 1946)
- Vickery, Hubert B, Ph D, Sc D (hon) Connecticut Agricultural Experiment Station, New Haven Lecturer in Physiological Chemistry, Yale University, Biochemist in Charge, Department of Biochemistry, Connecticut Agricultural Experiment Station, Member, National Academy of Sciences (2, 1923)
- Victor, Joseph, M D Camp Detrick, Frederick, Md Chief, Pathology Branch (4, 1935)
- Villee, Claude A, Jr, Ph D Harvard Medical School, Boston, Mass Assoc in Biological Chemistry (2, 1948)
- Virtue, Robert W, Ph D, M D 2134 E Iliff Ave, Denver 10, Colo Resident in Anesthesia, University of Iowa (2, 1939)
- Visscher, Frank E, M S, Ph D Upjohn Co, Kalamazoo 99, Mich Research Scientist, Department of Pharmacology and Endocrinology (1, 1947)
- Visscher, Maurice B, Ph D, M D University of Minnesota, Minneapolis Professor and Head of Dept of Physiology (1, 1927)
- Voegtlin, Carl, Ph D Sc D Dorset, Vt (1R, 1908, 2, 1908, 3, 1908)
- von Haam, Emmerich, M D Ohio State University, Columbus Professor of Pathology (4, 1938)
- Von Oettingen, W F, M D, Ph D National Institutes of Health, Industrial Hygiene Research Lab, Bethesda, Md Principal Industrial Toxicologist (3, 1925)
- Vorwald, Arthur J, Ph D, M D Saranac Lake, N Y Director of Research, The Edward L Trudeau Foundation and Director of the Saranac Laboratory (4, 1937)
- Vos, Bert J, Ph D, M D Division of Pharmacology, Food and Drug Administration, Washington, D C Associate Pharmacologist (3, 1941)
- Wachstein, Max, M D St Catherine's Hospital, Middleton, N Y Director of the Laboratory Research Assistant (4, 1947)
- Waddell, James, Ph D E I duPont de Nemours & Co, New Brunswick, N J Director of the Biological Laboratory (2, 1930, 5, 1935)
- Wadsworth, Augustus B, M D Manchester, Vermont (4, 1935, 6, 1920)
- Waelsch, Heinrich, M D, Ph D 722 West 168th St, New York 32, N Y Associate Research Biochemist, N Y State Psychiatric Institute and Hospital, Assistant Professor of Biological Chemistry, Columbia University (2, 1941)
- Wagman, Irving H, M A, Ph D Dept of Physiology, Jefferson Medical College, Philadelphia 7, Pa Associate in Physiology (1, 1946)
- Waisman, Harry A, M D, Ph D University of Illinois Research and Educational Hospital, Chicago, Ill Resident Fellow in Pediatrics (2, 1944)
- Wakeman, Alfred J, Ph D Hatfield Hill Road, Bethany, Conn Retired (2, 1906)
- Wakerlin, George E, Ph D, M D University of Illinois Medical School, 1853 W Polk St, Chicago Professor of Physiology (1, 1933, 3, 1934)
- Wakim, Khalil G, M D, Ph D Mayo Clinic, Rochester, Minn Consultant, Mayo Clinic and Professor of Physiology, Mayo Foundation (1, 1942)
- Walcott, William W, Ph D Department of Physiology, College of Physicians and Surgeons, Columbia University, New York 32, N Y Instructor (1, 1947)
- Wald, George, M A, Ph D Biological Laboratories, Harvard University, Cambridge, Mass (1, 1934)
- Walker, Arthur M, M D Veterans Administration Medicine & Surgery, Washington, D C (1, 1932, 3, 1939)
- Walker, Burnham S, Ph D, M D Boston University School of Medicine, 80 E Concord St, Boston, Mass Professor of Biochemistry (2, 1940)
- Walker, Harry A, Ph D Emory Univ School of Medicine, Emory University, Ga Asst Professor of Pharmacology (3, 1948)
- Walker, Sheppard M, M A, Ph D Washington University School of Medicine, St Louis, Mo Assistant Professor of Physiology (1, 1946)
- Wallen-Lawrence, Zonja, Ph D 4534 W Pine Blvd, St Louis, Mo (2, 1937)
- Walter, Annabel W 29 Perry St, New York 14, N Y Bacteriologist, New York City Dept of Health, Bureau of Labs (6, 1946)
- Walter, Carl W, M D Harvard Medical School, 25 Shattuck Street, Boston, Mass Director, Laboratory for Surgical Research, Assistant Professor of Surgery, Harvard Medical School, Senior Associate in Surgery, Peter Bent Brigham Hospital (4, 1942)
- Walters, Orville S, Ph D, M D McPherson, Kan Physician (1, 1936)
- Walton, Robert P, M A, Ph D, M D Medical College of the State of South Carolina, Charleston Professor of Pharmacology (3, 1933)
- Walton, Seth T, V M D, M S, Ph D Laboratory, Veteran's Hospital, Oteen, N C Director of Laboratories and Research (6, 1936)
- Walzer, Matthew, M D 20 Plaza St, Brooklyn,

- N Y *Attending in Allergy, Jewish Hospital of Brooklyn* (6, 1924)
- Wang, Chi Che, M S , Ph D U S Veterans Administration Hospital, Hines, Ill *In charge of Biochemical Research* (2, 1922, 5, 1933)
- Wang, Shih-Chun, M D , Ph D Columbia University College of Physicians and Surgeons, 630 W 168th St , New York City *Assistant Professor in the Department of Physiology* (1, 1943)
- Wangeman, Clayton P , B A , M D Broadway Medical Center, Associated Anesthesiologists, Seattle, Washington (3, 1946)
- Wangensteen, Owen Harding, M D University Hospital, Minneapolis 14, Minn *Professor of Surgery, University of Minnesota* (1, 1947, 4, 1931)
- Ward, Walter E , Ph D , M D Cutter Laboratories, Fourth and Parker Streets, Berkeley 1, Calif *Associate Medical Director* (6, 1947)
- Warner, Emory D , M D Medical Laboratories Bldg , Iowa City, Ia *Professor of Pathology* (4, 1937)
- Warner, Robert C , Ph D New York University College of Medicine, 477 First Ave , New York 16, N Y *Assistant Professor of Chemistry* (2, 1946)
- Warren, Charles O , Ph D , M D The Commonwealth Fund, 41 E 57th St , New York 22, N Y (1, 1941)
- Warren, James V , M D Emory University School of Medicine, Atlanta, Ga *Professor of Physiology, Associate Professor of Medicine* (1, 1947)
- Warren, Madeleine Field, A M , Ph D 9 High Rock St , Needham Mass Harvard School of Public Health, 55 Shattuck St , Boston, Mass *Associate in Physiology* (1, 1933)
- Warren, Marshall R , Ph D , M D Univ of Tennessee, College of Medicine, Memphis, Tenn *Asst Professor of Pharmacology* (3, 1948)
- Warren, Shields, M D 195 Pilgrim Rd , Boston, Mass *Pathologist, New England Deaconess Hospital, Assistant Professor of Pathology, Harvard Medical School* (4, 1929)
- Wartman, William Beckman, M D Northwestern Univ , 303 East Chicago Ave , Chicago 11, Ill *Morrison Professor of Pathology and Chairman of Dept* (4, 1940)
- Wasteneys, Hardolph, Ph D , F R S C University of Toronto, Toronto, Canada *Professor and Head of Department of Biochemistry* (2, 1915)
- Waterman, Robert E , B S Schering Corporation, 86 Orange St , Bloomfield, N J *Vice-President* (2, 1940)
- Waters, Ralph Milton, M D 1300 University Ave , Madison, Wis *Professor of Anesthesia, University of Wisconsin* (3, 1937)
- Watson, Cecil J , M D , Ph D Department of Medicine, University Hospital, Minneapolis, Minn *Professor and Head of Department of Medicine* (4, 1941)
- Watson, John B , A M , Ph D , LL D Box 526, Westport, Conn (1, 1907)
- Waud, Russell A , M D , M Sc , Ph D Medical School, University of Western Ontario, London, Ontario, Canada *Professor of Pharmacology* (1, 1925, 3, 1931)
- Waugh, David F , Ph D Department of Biology and Biological Engineering, Massachusetts Institute of Technology, Cambridge *Assistant Professor of Physical Biology* (1, 1913)
- Way, E Leong, M S , Ph D George Washington University School of Medicine, Washington, D C *Assistant Professor of Pharmacology* (3, 1947)
- Wearn, Joseph T , M D Lakeside Hospital, Cleveland, O *Professor of Medicine, Western Reserve University, Director of Medicine, Lakeside Hospital* (1, 1921)
- Weatherby, J H , M A , Ph D Dept of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va *Associate Professor of Pharmacology* (3, 1911)
- Weber, C J , M D , Ph D Veterans Administration Hospital, Wadsworth, Kan *Chief, Lab Service* (2, 1931)
- Webster, Bruce, M D , C M Cornell University Medical College, 1300 York Ave , New York City *Assistant Professor Medicine, Associate Attending Physician, New York Hospital* (5, 1935)
- Weed, Lewis H , A M , M D , Sc D National Research Council, 2101 Constitution Ave , Washington, D C (1, 1919)
- Wégria, René, M D Department of Medicine, Presbyterian Hospital, 622 W 168th St , New York City *Instructor in Medicine* (1, 1941)
- Weichert, Charles K , Ph D University of Cincinnati, Cincinnati, O *Professor of Zoology* (1, 1935)
- Weil, Alfred J , M D The Bronx Hospital, New York City *Director, Dept of Bacteriology* (6, 1940)
- Weil, Arthur, M D 952 5th Ave , New York, N Y (4, 1940)
- Weil, Leopold, Ph D Eastern Regional Research Laboratory, U S Department of Agriculture, Chestnut Hill Station, Philadelphia, Pa *Chemist* (2, 1942)
- Weinhouse, Sidney, Ph D Research Institute of Temple University, Philadelphia, Pa *Research Asso* (2, 1948)
- Weir, Everett G , M S , Ph D School of Medicine, Howard University, Washington, D C *Assistant Professor of Physiology* (1, 1941)
- Weiser, Russell S , Ph D Univ of Washington School of Med , St Louis, Mo *Assoc Professor of Immunology* (6, 1948)
- Weiss, Charles, M S , Ph D , M D Jewish Hospital

- tal, York & Tabor Roads, Philadelphia, Pa *Director of Laboratories* (4, 1934, 6, 1920)
- Weiss, Emil, M D , Ph D 5036 Bernard St , Chicago, Ill *Pathologist, People's Hospital* (6, 1927)
- Weiss, Paul, Ph D University of Chicago, Chicago, Ill *Professor of Zoology* (1, 1936)
- Welch, Arnold D , Ph D , M D Western Reserve University School of Medicine, Cleveland, O *Professor of Pharmacology* (3, 1942, 5, 1944)
- Welch, Henry, M S , Ph D Rm 6171 S Agriculture Bldg , Washington, D C *Chief, Division of Penicillin Control and Immunology, U S Food and Drug Administration* (6, 1932)
- Weld, Charles Beecher, M A , M D Dalhousie University, Halifax, N S , Canada *Professor of Physiology* (1, 1936)
- Weld, Mrs Julia T College of Physicians and Surgeons, 630 W 168th St , New York City *Research Associate in Pathology* (6, 1920)
- Welker, William H , Ph D , D Sc 1853 W Polk St , Chicago, Ill *Professor Emeritus of Biological Chemistry, College of Medicine, University of Illinois* (2, 1906)
- Weller, Carl Vernon, M D 1130 Fair Oaks Parkway, Ann Arbor, Mich *Professor of Pathology and Chairman, Department of Pathology, University of Michigan* (4, 1923)
- Wells, Herbert S , M D University of Minnesota Minneapolis 14 *Professor of Clinical Physiology* (1, 1932)
- Wells, Joseph Albert, M S , Ph D Northwestern University Medical School, Chicago, Ill *Associate in Pharmacology* (3, 1944)
- Welsh, John H , Ph D Biological Laboratories, Harvard University, 16 Divinity Ave , Cambridge 38, Mass *Associate Professor of Zoology* (1, 1945)
- Wendel, William B , Ph D Tulane University School of Medicine, New Orleans, La *Professor of Biochemistry and Head of Dept* (2, 1932)
- Werber, Erna A , Ph D 44 W 83rd St , New York City *Director, Research Lab, Jewish Hospital of Brooklyn* (6, 1948)
- Werkman, C H , Ph D , D Sc Science Hall, Iowa State College, Ames *Professor and Head of Department of Bacteriology* (2, 1942)
- Werle, Jacob M , M D , Capt , 138 Evacuation Hospital, A P O 408, New York, N Y *Neurosurgeon* (1, 1943)
- Werner, Harold W , Ph D The Wm S Merrell Co , Lockland Station, Cincinnati, O *Director of Pharmacology Research* (3, 1942)
- Wertenberger, Grace E , S M Ph D Women's Medical College of Pennsylvania Philadelphia *Assistant Professor of Physiology* (1, 1943)
- Werthessen, Nicholas T , Ph D Shrewsbury, Mass Worcester Foundation for Experimental Biology *Senior Fellow* (1, 1946)
- Wesson, Laurence Goddard, Ph D Forsyth Dental Infirmary, Boston, Mass *Research Biochemist* (2, 1929, 3, 1932)
- West, Edward S , M S , Ph D University of Oregon Medical School, Portland *Professor of Biochemistry* (2, 1925)
- West, Harold D , M S , Ph D Meharry Medical College, Nashville 8, Tenn *Professor of Biochemistry and Head of Dept of Biochemistry* (2, 1946)
- West, Randolph, M D Columbia Univ College of Physicians and Surgeons, New York City *Professor of Medicine* (2, 1931)
- Westerfeld, Wilfred Wiedey, Ph D Syracuse University College of Medicine, Syracuse 10, N Y *Professor of Biochemistry* (2, 1944)
- Weston, Raymond E , M D , Ph D Medical Division, Montefiore Hospital, New York 67, N Y *Assistant in Medicine* (1, 1947)
- Weymouth, Frank W , Ph D Stanford University, Calif *Professor of Physiology and Executive of the Department* (1, 1917)
- Wheeler, George W , M D New York Hospital, 235 E 73rd St , New York City *Assistant Director* (6, 1920)
- Wheeler, Mary W , M A Division of Laboratories and Research, New York State Department of Health, Albany *Associate Bacteriologist* (6, 1933)
- Wheelon, Homer, M S , M D University of Washington, Seattle, Wash *Prof of Medicine* (1, 1919)
- Whipple, George H , M D , Sc D University of Rochester, Rochester, N Y *Professor of Pathology and Dean of the School of Medicine and Dentistry, Member of the National Academy of Sciences* (1, 1911, 4, 1913)
- White, Abraham, M A , Ph D 333 Cedar St , New Haven, Conn *Associate Professor of Physiological Chemistry, Yale University School of Medicine* (2, 1934, 5, 1937)
- White, Florence R , M A , Ph D National Cancer Institute, Bethesda 14, Md *Biochemist, National Institute of Health* (2, 1946)
- White, Frank D , Ph D , F R I C University of Manitoba, Faculty of Medicine, Winnipeg, Canada *Professor of Biochemistry* (2, 1931)
- White, Harvey Lester, M D Washington University, Medical School, St Louis 10, Mo *Professor of Physiology* (1, 1923)
- White, Julius, A M , Ph D National Cancer Institute, Bethesda, Md *Head Chemist* (2, 1937)
- White, Paul Dudley, M D , Massachusetts General Hospital, Boston *Lecturer in Medicine, Harvard Medical School, Physician (in charge of Cardiac Clinics and Laboratory), Mass General Hospital* (3, 1921)
- Whitehead, Richard W , M A , M D University of Colorado School of Medicine, 4200 E Ninth

- Ave, Denver *Professor of Physiology and Pharmacology* (1, 1933, 3, 1928)
- Whitehorn, William V, M D University of Illinois College of Medicine, Chicago *Assistant Professor of Applied Physiology* (1, 1947)
- Wiener, Alexander S, M D 64 Rutland Rd, Brooklyn, N Y *Bacteriologist and Serologist to Office of Chief Medical Examiner of New York City, Head of Transfusion Division, Jewish Hospital of Brooklyn* (6, 1932)
- Wiersma, Cornelis A G, M A, Ph D California Institute of Technology, Pasadena *Associate Professor of Physiology* (1, 1941)
- Wiggers, Carl J, M D, Sc D Medical School, Western Reserve University, Cleveland, O *Professor and Director of Physiology* (1, 1907, 3R, 1909)
- Wiggers, Harold C, Ph D Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany 3, N Y *Professor and Chairman of Department* (1, 1938)
- Wigodsky, Herman S, Ph D, M D Division of Medical Sciences, Committee on Atomic Casualties, National Research Council, Washington 25, D C (1, 1943)
- Wikler, Abraham, M D U S Public Health Service Hospital, Lexington, Ky *Surgeon (R), U S Public Health Service* (3, 1944)
- Wilber, Charles G, M A, Ph D The Biological Laboratory, Fordham University, New York 58, N Y *Assistant Professor of Physiology* (1, 1947)
- Wilde, Walter S, Ph D Tulane University of Louisiana, School of Medicine, New Orleans, La *Associate Professor of Physiology Associate Member* (1, 1944)
- Wilder, Russell M, Ph D, M D Mayo Clinic, Rochester, Minn *Professor of Medicine, Mayo Foundation, University of Minnesota* (1, 1921, 4, 1924, 5, 1933)
- Wiley, Frank H, M S, Ph D Food and Drug Administration, Federal Security Agency, Washington 25, D C *Chemist* (2, 1933)
- Wilhelein, Jane Russell, Ph D Yale University School of Medicine, 333 Cedar St, New Haven, Conn *Instructor in Physiological Chemistry* (1, 1939)
- Wilhelm, Alfred E, Ph D 333 Cedar St, New Haven, Conn Yale University School of Medicine *Assistant Professor of Physiological Chemistry* (2, 1942)
- Wilhelmj, Charles Martel, M D Creighton University School of Medicine, Omaha, Neb *Professor of Physiology* (1, 1931)
- Wilkerson, Vernon A, M D, Ph D Medical Arts Bldg, 611 I St, NW, Washington, D C *Biochemical Consultant* (2, 1936)
- Williams, Carroll M, A M, Ph D, M D Biological Laboratories, Harvard University, Cambridge, Mass *Assistant Professor of Zoology* (1, 1947)
- Williams, Edwin G, M D, D T M, D T H National Institute of Health, Bethesda 14, Md *Senior Surgeon U S Public Health Service, Director of Research, U S P H S Hospital, Lexington, Ky* (3, 1944)
- Williams, Harold H, Ph D Savage Hall, Cornell University, Ithaca, N Y *Professor of Biochemistry* (2, 1938, 5, 1936)
- Williams, Horatio B, M D, Sc D Box 893, Greenwich, Conn *Dalton Professor of Physiology Emeritus, Columbia University* (1, 1912)
- Williams, J W, M S, Ph D University of Wisconsin, Chemistry Bldg, Madison *Professor of Chemistry* (2, 1944)
- Williams, Ray D, M S, M D 6834 Waterman St, St Louis, Mo *Assistant Professor of Clinical Medicine, Washington University* (5, 1941)
- Williams, Robert Hardin, M D Dept of Medicine, Univ of Washington, Seattle *Professor and Executive Officer* (4, 1940)
- Williams, Robert R, M S, D Sc 297 Summit Ave, Summit, N J *Director of Grants, Research Corp* (2, 1919, 5, 1941)
- Williams, Roger J, Ph D, D Sc University of Texas, Department of Chemistry, Austin *Professor of Chemistry, Director, Biochemical Institute* (2, 1931)
- Williams, W Lane, M A, Ph D University of Minnesota, Medical School, Minneapolis 14, Minn *Assistant Professor of Anatomy* (1, 4, 1947)
- Wills, J H, M S, Ph D Pharmacology Section, Medical Division, Army Medical Center, Md (1, 1943)
- Wilson, David Wright, M S, Ph D University of Pennsylvania Medical School, Philadelphia *Benjamin Rush Professor of Physiological Chemistry* (1, 1915, 2, 1915)
- Wilson, Frank N, M D University Hospital, Ann Arbor, Mich *Professor of Medicine, University of Michigan* (4, 1925)
- Wilson, John W, Ph D Wright-Patterson Air Force, Aero Medical Lab, Dayton, Ohio *Res Physiologist* (1, 1948)
- Wilson, Karl M, M D University of Rochester, School of Medicine, Rochester, N Y *Professor of Obstetrics and Gynecology* (4, 1927)
- Wilson, P W, Ph D Department of Agricultural Bacteriology, University of Wisconsin, Madison *Professor in Agricultural Bacteriology* (2, 1939)
- Wilson, Robert H, Ph D U S Dept of Agriculture, Western Regional Research Laboratory, 800 Buchanan St, Albany, Calif *Pharmacologist* (3, 1937)
- Winder, Claude V, Sc D Parke Davis and Co,

- Detroit, Mich *Research Pharmacologist* (1, 1938, 3, 1948)
- Windle, William Frederick, Ph D, Sc D *Medical School, University of Pennsylvania, Philadelphia Professor of Anatomy and Chairman of Dept of Anatomy* (1, 1937)
- Winkler, Walter LaF, M D 1014 St Paul St, Baltimore, Md *Associate in Medicine, Johns Hopkins Medical School* (6, 1938)
- Winnick, Theodore, Ph D *Division of Biochemistry, Univ of California, Berkeley, Calif Research Associate* (2, 1946)
- Winter, Charles A, Ph D *Merck Institute for Therapeutic Research, Rahway, N J Research Associate* (1, 1940)
- Winter, Irwin Clinton, Ph D, M D G D Searle & Co, P O Box 5110, Chicago 80, Ill *Director of Clinical Research* (3, 1941)
- Winters, Jet C, M A, Ph D *University of Texas, Austin Professor of Home Economics* (5, 1933)
- Winternitz, M C, M D *Yale University School of Medicine, New Haven, Conn Anthony N Brady Professor of Pathology* (4, 1913)
- Wintersteiner, Oskar, Ph D *The Squibb Institute for Medical Research, New Brunswick, N J Member, Head, Division of Organic Chemistry, Honorary Professor of Biochemistry, Rutgers University* (2, 1930)
- Wintrobe, Maxwell Myer, M D, Ph D *University of Utah School of Medicine, Salt Lake City Professor and Head of the Department of Internal Medicine, Director, Laboratory for the Study of Hereditary and Metabolic Disorders* (4, 1940)
- Winzler, Richard J, Ph D *Dept of Biochemistry, Univ of Southern Calif Medical School, Los Angeles 7, Calif Associate Professor of Biochemistry* (2, 1946)
- Wiseman, Bruce Kenneth, M D *Kinsman Hall, Ohio State University, Columbus Professor and Chairman of Department of Medicine, Assistant Director of Medical Research* (4, 1932)
- Wislocki, George B, M D *Harvard University Medical School, 25 Shattuck St, Boston, Mass Parkman Professor of Anatomy* (1, 1924)
- Witebsky, Ernest, M D *Buffalo General Hospital, 100 High St, Buffalo, N Y Professor and Head, Dept of Bacteriology and Immunology* (6, 1935)
- Wittich, Fred W, M D 401 LaSalle Medical Bldg Minneapolis 2, Minn *Sec-Treas American College of Allergists, Chairman, Executive Committee, International Association of Allergists* (6, 1944)
- Wolbach, S Burt, M D *Children's Hospital, Boston, Mass Shattuck Professor of Pathological Anatomy, Emeritus, Harvard Medical School, Director of Nutritional Research, Children's Hospital* (4, prior to 1920)
- Wolf, Abner, M D *Columbia Univ Coll of Physicians and Surgeons, New York City Assoc Professor of Neuropathology* (4, 1948)
- Wolf, Arnold Veryl, Ph D *Union University, Albany Medical College, Albany, N Y Associate Professor of Physiology and Pharmacology* (1, 1946)
- Wolf, Stewart, M D *Cornell Univ Med Coll, New York City Asst Professor of Medicine* (1, 1948)
- Wolff, Harold G, M D, M A *New York Hospital, 525 E 68th St, New York City Associate Professor of Medicine, Cornell University Medical College, Associate Attending Physician, New York Hospital* (1, 1930, 3, 1942)
- Wolff, William A, M A, Ph D *Bowman Gray School of Medicine, Winston Salem 7, N C Assistant Professor of Biochemistry* (2, 1947)
- Womack, Madelyn, Ph D *Foods and Nutrition Division, Agricultural Research Administration, U S Department of Agriculture, Washington 25, D C Biochemist* (5, 1947)
- Wood, Earl H, Ph D, M D *Mayo Aero medical Unit, Mayo Foundation, Rochester, Minn Assoc Professor of Physiology, Mayo Foundation, Graduate Sch, Univ of Minnesota, Consultant in Physiology, Mayo Clinic* (1, 1943, 3, 1948)
- Wood, Harland G, Ph D *Department of Biochemistry, Western Reserve University, Cleveland, Ohio Professor of Biochemistry* (2, 1944)
- Wood, John L, Ph D *University of Tennessee School of Biological Sciences, 875 Monroe Avenue, Memphis 3, Tenn Associate Professor of Chemistry* (2, 1947)
- Woodbury, Robert A, Ph D, M D *Department of Pharmacology, University of Tennessee, Memphis Professor of Pharmacology* (1, 1936, 3, 1941)
- Woods, Alan C, M D *Wilmer Institute, Johns Hopkins Hospital, Baltimore, Md Ophthalmologist-in-Chief, Acting Professor of Ophthalmology, Johns Hopkins University, Director, Wilmer Ophthalmological Institute* (6, 1918)
- Woods, Ella, A M, Ph D *University of Idaho, Moscow Home Economist, Experiment Station* (2, 1925, 5, 1933)
- Woodward, Alvalyn E, M S, Ph D *University of Michigan, Ann Arbor Assistant Professor of Zoology* (1, 1932)
- Woodyatt, Rollin T, M D 237 E Delaware Place, Chicago, Ill *Professor of Medicine, Rush Medical College, University of Chicago* (2, 1912)
- Woolley, D Wayne, Ph D *Rockefeller Institute*

- for Medical Research, New York City *Member* (2, 1946, 5, 1941)
- Woolpert, Oram C, M D, Ph D Camp Detrick, Frederick, Md *Technical Director, Biological Division, Chemical Corps* (6, 1947)
- Woolsey, Clinton N, M D Univ of Wisconsin, Service Memorial Institutes, Madison, Wis *Charles Sumner Slichter Research Prof of Neurophysiology* (1, 1938)
- Wortis, S Bernard, M D Department of Psychiatry, New York University College of Medicine, New York 16 *Professor of Psychiatry and Chairman of the Department, Director, Psychiatric Division of Bellevue Hospital* (1, 1947)
- Wright, Angus, M D University of Southern California Medical School, 657 S Westlake Ave, Los Angeles *Pathologist, California Hospital* (4, 1935)
- Wright, Arthur W, M D Albany Medical College, New Scotland Ave, Albany, N Y *Professor of Pathology and Bacteriology* (4, 1941)
- Wright, Charles Ingham, M S, Ph D National Institute of Health, Bethesda, Md *Senior Pharmacologist, U S Public Health Service* (1, 1935, 3, 1936)
- Wright, George G, Ph D Camp Detrick, Frederick, Md *Chief, Special Procedures Branch, S-Division* (6, 1943)
- Wright, Harold N, M S, Ph D University of Minnesota, Minneapolis *Associate Professor of Pharmacology* (3, 1933)
- Wright, Lemuel D, M S, Ph D Medical Research Division, Sharp and Dohme, Inc, Glenside, Pa *Director of Nutritional Research* (2, 1946, 5, 1946)
- Wright, Sydney L, M A, Ph D Endsmeet Farm, Wyncote, Pa (2, 1933)
- Wyckoff, Ralph W G, Ph D U S Public Health Service, National Institute of Health, Bethesda, Md *Senior Scientist* (6 1940)
- Wyman, Jeffries, Jr, Ph D Biological Laboratories, Harvard University, Cambridge, Mass *Associate Professor of Zoology* (1, 1928)
- Wyman, Leland C, Ph D 5 Furnival Rd, Jamaica Plain 30, Mass Boston University School of Medicine, Boston, Mass *Associate Professor of Physiology* (1, 1927)
- Wynne, Arthur M, M A, Ph D, F R S C Department of Biochemistry, University of Toronto, Toronto, Canada *Professor of Biochemistry* (2, 1940)
- Yonkman, Frederick F, Ph D, M D Ciba Pharmaceutical Products, Inc, Summit, N J *Director of Research at Ciba and Lecturer in Pharmacology, College of Physicians and Surgeons, Columbia University, New York City* (3, 1931)
- Youmans, John B, M D Univ of Illinois, Chicago, Ill *Dean and Professor of Medicine, College of Medicine, Medical Director, Research and Educational Hospitals* (5, 1948)
- Youmans, William Barton, M A, Ph D, M D University of Oregon Medical School, Portland *Professor of Physiology* (1, 1939)
- Young, A G, Ph D, M D 520 Commonwealth Ave, Boston, Mass *Assistant Professor of Therapeutics, Boston University School of Medicine, Medical Director, Corey Hill Hospital, Brookline* (3, 1925)
- Young, E G, Ph D, F R S C Dalhousie University, Halifax, N S, Canada *Professor of Biochemistry* (2, 1925)
- Youngburg, Guy E, M S, Ph D 700 N Main St, Hutchinson, Kansas (2, 1927)
- Yuile, Charles L, M D, C M University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd, Rochester, N Y *Associate Professor of Pathology* (4, 1941)
- Zechmeister, L Dr Ing (Zurich) California Institute of Technology, Pasadena *Professor of Organic Chemistry* (2, 1941)
- Zeckwer, Isolde T, M D School of Medicine, University of Pennsylvania, Philadelphia *Assistant Professor of Pathology* (1, 1934, 4, 1927)
- Zeldis, Louis Jenrette, M D Brookhaven, Long Island, N Y (4, 1945)
- Zimmerman, Harry M, M D Montefiore Hospital, Gun Hill Rd, New York 67, N Y (4, 1933)
- Zirkle, Raymond E, Ph D Univ of Chicago, Chicago, Ill *Professor of Botany and Director, Inst of Radiobiology and Biophysics* (1, 1948)
- Zittle, Charles A, Ph D U S Dept of Agriculture, Eastern Regional Res Lab, Philadelphia, Pa *Chemist, Protein Div* (2, 1946)
- Zucker, Marjorie B Ph D Department of Physiology, College of Physicians and Surgeons, Columbia University (1, 1947)
- Zweifach, Benjamin W, Ph D Department of Medicine, New York Hospital and Cornell University Medical College, 525 E 68th St, New York 21 *Asst Prof of Physiology* (1, 1945)
- Zwemer, Raymund L, Ph D 5003 Battery Lane, Bethesda 14, Md National Academy of Sciences, 2101 Constitution Ave, Washington 25, D C *Executive Secretary, National Academy of Sciences and National Research Council* (1, 1930)



## SUMMARY OF MEMBERSHIP

|   |      |
|---|------|
| The American Physiological Society                              | 1090 |
| American Society of Biological Chemists                         | 794  |
| American Society for Pharmacology and Experimental Therapeutics | 386  |
| The American Society for Experimental Pathology                 | 328  |
| American Institute of Nutrition                                 | 333  |
| The American Association of Immunologists                       | 321  |
| Total members by Societies                                      | 3152 |

## DECEASED MEMBERS

1948

|   |  |
|---|--|
| Auer, John (1, 3) April 30, 1948          | Lipschitz, Lindley Werner (3) February 1, 1948 |
| Austin, Richard Sisson (4) April 30, 1948 | McClosky, William T (3) January 12, 1948       |
| Burge, W E (1) March 28, 1948             | Myers, Victor C (1, 2, 5) October 9, 1948      |
| Conrad, Ralph M (2) May 22, 1948          | Nye, Robert N (6)                              |
| Ellis, Frederick W (1) April 30, 1948     | Rose, Anton R (2, 5) Sept 23, 1948             |
| Favorite, Grant O (6) May 1948            | SubbaRow, Y (2) August 9, 1948                 |
| Haterius, Hans O (1)                      | Wallace, George B (1, 2, 3) January 15, 1948   |
| Hunt, Reid (1, 2, 3) March 11, 1948       | Wastl, Helene (1)                              |
| Koch, Fred Conrad (2, 5) January 26, 1948 | Wheeler, Kenneth M (6)                         |
| Wheeler, Ruth (2, 5) September 29, 1948   |  |



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